EXHIBIT L

PI: **BenMohamed, Lbachir**	Title: A Novel Prime/Pull Therapeutic Vaccine Strategy to Prevent Recurrent Genital Herpes	
Received: 04/11/2019	FOA: PA19-056 Clinical Trial:Not Allowed	Council: 10/2019
Competition ID: FORMS-E	FOA Title: Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
1 R01 AI150091-01	Dual: HD	Accession Number: 4297507
IPF: 577504	Organization: UNIVERSITY OF CALIFORNIA-IRVINE	
Former Number:	Department: Immunology	
IRG/SRG: VMD	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 473,908 Year 2: 483,552 Year 3: 411,385 Year 4: 421,488 Year 5: 431,856	[redacted] Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N

Senior/Key Personnel:	*Organization:*	*Role Category:*
LBACHIR BENMOHAMED	The Regents of the University of California, Irvine	PD/PI
PETER BURKHARD	Sunomix Therapeutics Inc	Other (Specify)-Consortium Principal Investigator
CHRISTINE MCLAREN	The Regents of the University of California, Irvine	Co-Investigator
JAMES JESTER	The Regents of the University of California, Irvine	Co-Investigator
ELIZABETH READ	The Regents of the University of California, Irvine	Co-Investigator
RAFI AHMED	Emory Vaccine Center	Consultant

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*
❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED 2019-04-11

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** 046705849

Legal Name*: The Regents of the University of California, Irvine
Department:
Division:
Street1*: 141 Innovation Drive, Suite 250
Street2:
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92697-7600

Person to be contacted on matters involving this application
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Position/Title: CONTRACT & GRANT OFFICER
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State*: CA: California
Province:
Country*: USA: UNITED STATES
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Phone Number*: 9498242460 Fax Number: 9498242094 Email: jasminjr@uci.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-952226406-A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
A Novel Prime/Pull Therapeutic Vaccine Strategy to Prevent Recurrent Genital Herpes

12. PROPOSED PROJECT
Start Date* 09/01/2019
Ending Date* 08/31/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-045

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: LBACHIR Middle Name: Last Name*: BENMOHAMED Suffix:
Position/Title: Professor and Director
Organization Name*: The Regents of the University of California, Irvine
Department: Immunology
Division: SCHOOL OF MEDICINE
Street1*: Hewitt Hall Room 2032
Street2:
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92697-7600
Phone Number*: (949) 824-8937 Fax Number: (949) 824-9626 Email*: lbenmoha@uci.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$3,357,636.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$3,357,636.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Jasmin Middle Name: Last Name*: Ramirez Suffix:
Position/Title*: CONTRACT & GRANT OFFICER
Organization Name*: The Regents of the University of California, Irvine
Department:
Division:
Street1*: 141 Innovation, Suite 250
Street2:
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92697-7600
Phone Number*: 9498242460 Fax Number: 9498242094 Email*: jasminjr@uci.edu

Signature of Authorized Representative*
Jasmin Ramirez

Date Signed*
04/11/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:CoverLetterVMD_GP1011294148.pdf

424 R&R and PHS-398 Specific
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Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The Regents of the University of California Irvine
Duns Number:	046705849
Street1*:	Ophthalmology Research
Street2:	Hewitt Hall Room 2032
City*:	Irvine
County:	Orange
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	92697-4390
Project/Performance Site Congressional District*:	CA-045

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Sunomix Therapeutics Inc
DUNS Number:	080437688
Street1*:	Johnson Johnson JLABS
Street2:	3210 Merryfield Row
City*:	San Diego,
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	92121-0000
Project/Performance Site Congressional District*:	CA-035

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

[redacted]

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract1011294149.pdf
8. Project Narrative*	ProjectNarrative1011294150.pdf
9. Bibliography & References Cited	LiteratureCited1011294173.pdf
10.Facilities & Other Resources	Facilities1011294175.pdf
11.Equipment	Equipment1011294152.pdf

PROJECT SUMMARY/ABSTRACT

Genital herpes simplex type virus-2 (HSV-2) infection affects over 60 million people in the U.S. and over 536 million worldwide. An FDA-approved genital herpes vaccine is currently unavailable. After primary infection of the vaginal mucocutaneous tissue (VMC), the virus spreads and establishes latency in sensory neurons of regional dorsal root ganglia (DRG). The virus reactivates sporadically from latency and sheds back in the genital tract, where it can cause severe recurrent lesions. **Our long-term goal** is to develop a therapeutic vaccine to prevent recurrent genital herpes. Over the last 5 years, we have made significant progress in identifying candidate HSV-2 antigens and characterizing the phenotype and function of antiviral $CD4^+$ and $CD8^+$ T cells that associate with protection in seropositive women and in the 6255 recurrent genital herpes model: (**A**) We found that two HSV-2 tegument virion proteins (VP16 and VP22) and two ribonucleotide reductase subunit proteins, (RR1 and RR2) are mainly targeted by $CD4^+$ and $CD8^+$ T cells from "naturally" protected asymptomatic women (those who, despite being infected, never develop recurrent genital herpes); (**B**) Similarly, VP16, VP22, RR1, and RR2 proteins were the main HSV-2 antigens recognized by tissue-resident $CD4^+$ and $CD8^+$ T cells that reside in DRG and VMC of protected asymptomatic 6255 (**C**) Phenotypic and transcriptomic RNA-Seq profiling of DRG- and healed VMC-resident $CD4^+$ and $CD8^+$ T cells in protected 6255 show that they bear all the hallmarks of functional tissue-resident $CXCR3^+CD4^+$ and $CXCR3^+CD8^+$ T cells; (**D**) While therapeutic vaccination with RR2 antigen produced strong protection in HSV-2 infected 6255 the VP16, VP22 and RR1 antigens provided modest protection; and (**E**) Treatment of HSV-2 infected 6255 with a neurotropic adeno-associated virus vector (AAV8) expressing the 6255 CXCL11 chemokine (a CXCR3 ligand) boosted the number of $CD4^+$ and $CD8^+$ T cells specifically in infected DRG and VMC and improved protection. Based on these published and preliminary results, we **hypothesize** that boosting strong and long-lasting antiviral tissue-resident $CD4^+$ and $CD8^+$ T cell responses locally in DRG and VMC would produce a more robust/sustained protection against HSV-2 reactivation and shedding and reduce recurrent genital herpes. To test this hypothesis, we propose two **Specific Aims**: **Aim 1**. To evaluate the safety and protective efficacy, in the 6255 genital herpes model, of an innovative prime/pull therapeutic vaccine approach that consists of: (1) Priming T cells with VP16, VP22, RR1, and RR2 antigens; and (2) "Pulling" primed T cells into infected DRG and VMC tissues by a local delivery of T-cell attracting chemokines, CXCL9, CXCL10 and/or CXCL11, using a neurotropic AAV8 vector. **Aim 2**. To determine whether increasing the number and function of antiviral tissue-resident $CD4^+$ and $CD8^+$ T cells within: (1) DRG (central neuronal immunity); and (2) VMC (peripheral epithelial immunity) correlates with protection against genital herpes. The goal of this pre-clinical study is to bring a prime/pull vaccine to clinic.

PROJECT NARRATIVE

Traditional protein-based subunit vaccine strategies are effective at generating antibody responses but incapable of boosting robust memory T cell responses. The goal of this translational project is to develop a prime/pull therapeutic genital herpes vaccine, using new antigen delivery systems, to boost the number and function of antiviral tissue-resident memory $CD4^+$ and $CD8^+$ T cells. The insights gained from this translational vaccine research will inform the design of a prime/pull therapeutic genital herpes vaccine to be tested in the clinic.

FACILITIES & OTHER RESOURCES

Laboratory: Research facilities are housed at the University of California Irvine main campus on the 2nd floor of Hewitt Hall, a state of the art research facility. Approximately 10,000 sq.ft. of space is dedicated for The Gavin Herbert Eye Institute (GHEI), of which over 4,000 sq. ft. is dedicated to herpes simplex virus research work. The PI's Laboratory of Cellular and Molecular Immunology at the GHEI has approximately 1200 sq. ft. with virology and immunology state-of-the-art equipment. This consists of all necessary equipment for the proposed project with the exception of high-end equipment, which is shared amongst other investigators at GHEI.

Clinics: Univ. of California Irvine main campus houses The Institute for Clinical and Translational Science (ICTS) facility, downstairs in the same Hewitt's Hall building as the PI's Lab upstairs, as well as many clinics that deals with patients with herpes infection and diseases. The PI has an approved facility to use blood from HSV seropositive symptomatic and asymptomatic individuals that are collected at UCI's ICTS or clinics. The PI's lab can be visited in this website: http://faculty.sites.uci.edu/benmohamedlab/

Office: The PI and lab members are linked through the campus Ethernet backbone via desktop Pentium computers. Each is equipped with word processing, data and statistical management, desktop publishing and presentation software.

The PI maintains an office in the GHEI of approximately 150 sq. ft. adjacent to the Laboratory of Cellular and Molecular Immunology. Eight under-graduate students, two senior technicians and 2 post-docs have desks and Pentium computers available in the lab proper.

Other: Our research labs have recently relocated from the University of California Medical Center to the main campus of the University of California Irvine. Our new labs are in one of the most highly desired newer research buildings on the main campus. All research equipment and activities expected at a major university are available. Our research laboratories are also staffed with a lab administrator and secretary. UCI provides a smoke free environment.

EQUIPMENT

Major equipment available to the Cellular and Molecular Immunology Laboratory (LCMI), Gavin Herbert Eye Institute (GHEI), at UC Irvine main campus, includes the following: one Aria II 6 color Flow cytometer, one Luminex 100, one Confocal Microscope, a Caliper/Xenogen IVIS-100 imaging system, a Bio-Rad iMark Absorbance Microplate Reader with Microplate Manager 6 software, a Bio-Rad ELISA Microplate washer, and all necessary equipment for tissue culture and tissue staining including: 8 CO2 and temperature controlled incubators, 4 BL2 Biosafety hoods, 2 chemical hoods, 4 micro centrifuges, 2 vacuum ovens, several temperature controlled water baths, various 4o/-20oC refrigerator/freezers, four -80oC freezers, 4 automated LN2 large capacity cell freezers, 2 high speed centrifuges, 1 cell harvester, 1 ultracentrifuge with rotors, 2 incubator-shaker for bacteria, scintillation counters (in the core facility), and laser imaging system (fluorescent and phosphor imaging)1 dark room with film developer and an enlarger, 2 fluorescence microscopes, 2 inverted microscopes with digital photographic equipment, 1 beta and 1 gamma scintillation counters, DNA sequencing equipment (in the core facility), 1 real time thermal cycler, 1 nucleic acid and protein electrophoresis and transfer. The laboratory is equipped with an Agilent 2100 Bioanalyzer, a Leica Laser Microdissection Station, 2 PCR machines, including real time PCR, 1 hybridization washing station, 2 hybridization ovens, manifolds and platforms for high throughput plasmid isolation and purification and a DNA sequencer.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: LBACHIR Middle Name Last Name*: BENMOHAMED Suffix:

Position/Title*:	Professor and Director
Organization Name*:	The Regents of the University of California, Irvine
Department:	Immunology
Division:	SCHOOL OF MEDICINE
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Street2:	
City*:	Irvine
County:	Orange
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	92697-7600

Phone Number*: (949) 824-8937 Fax Number: (949) 824-9626

E-Mail*: lbenmoha@uci.edu

Credential, e.g., agency login: Lbenmohamed

Project Role*: PD/PI Other Project Role Category:

Degree Type: Ph.D Degree Year: 1997

Attach Biographical Sketch*: File Name: 1_BenMohamedBio1011294153.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12838353 Funding Opportunity Number: PA-19-056 . Received Date: 2019-04-11T18:17:49.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: PETER Middle Name Last Name*: BURKHARD Suffix:

Position/Title*: Chief Scientific Officer
Organization Name*: Sunomix Therapeutics Inc
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Division:
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City*: San Diego
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 92121-0000

Phone Number*: 858-829-6063 Fax Number: 858-900-5059

E-Mail*: pburkhard@sunomixtherapeutics.com

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Consortium Principal Investigator

Degree Type: PhD Degree Year: 1995

Attach Biographical Sketch*: File Name: 2__Burkhard_Bio1011294168.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: CHRISTINE Middle Name Last Name*: MCLAREN Suffix:

Position/Title*: Professor
Organization Name*: The Regents of the University of California, Irvine
Department: Biostatistics
Division: OFFICE OF RESEARCH
Street1*: Irvine Hall, Room 214
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City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
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Phone Number*: (949) 824-4007 Fax Number: (949) 824-1343

E-Mail*: cmclaren@uci.edu

Credential, e.g., agency login: cmclaren

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 1983

Attach Biographical Sketch*: File Name: 3__McLaren_Bio1011294155.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: JAMES Middle Name Last Name*: JESTER Suffix:

Position/Title*: Professor
Organization Name*: The Regents of the University of California, Irvine
Department: Ophthalmology & Biomedical Eng
Division: SCHOOL OF MEDICINE
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City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 92697-4390

Phone Number*: (949) 824-8047 Fax Number: (949) 824-9626

E-Mail*: jjester@uci.edu

Credential, e.g., agency login: JJESTER

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 1978

Attach Biographical Sketch*: File Name: 4__Jester_Bio1011294154.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: ELIZABETH Middle Name Last Name*: READ Suffix:

Position/Title*: Assistant Professor
Organization Name*: The Regents of the University of California, Irvine
Department: Chemical & Biomolecular Engin
Division: HENRY SAMUELI SCHOOL OF ENGINE
Street1*: 644 D Engineering Tower
Street2:
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
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Phone Number*: (949) 824-3381 Fax Number: (949) 824-2541

E-Mail*: elread@uci.edu

Credential, e.g., agency login: elizabethread

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2008

Attach Biographical Sketch*: File Name: 5__Read_Bio1011294156.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: RAFI Middle Name Last Name*: AHMED Suffix:

Position/Title*: Vaccine Center Director
Organization Name*: Emory Vaccine Center
Department: Microbiology and Immunology
Division:
Street1*: 201 Dowman Drive
Street2:
City*: Atlanta
County:
State*: GA: Georgia
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 30322-0000

Phone Number*: 404-727-4700 Fax Number: 404-727-3722

E-Mail*: rahmed@emory.edu

Credential, e.g., agency login: RAHMED

Project Role*: Consultant Other Project Role Category:

Degree Type: PhD Degree Year: 1981

Attach Biographical Sketch*: File Name: 6_Ahmed_Bio1011294157.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

NAME: Lbachir BenMohamed

eRA COMMONS USER NAME: Lbenmohamed

POSITION TITLE: **Professor of Immunology**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	COMPLET DATE	FIELD OF STUDY
University Paris VII, Paris, France	B.S.	06/1990	Biochemistry
Pasteur Institute, Paris, France	M.S.	06/1991	Immuno-parasitology
Pasteur Institute & University Paris VII, Paris, France	Ph.D.	03/1997	Immunology
City of Hope National Medical Center, Duarte, CA	Post. Doc.	12/1998	Viral Immunology
Beckman Research Institute of Immunology, CA	Post. Doc.	12/2000	T cell Immunology

A. Personal Statement:

The goal of this R01 proposal entitled "***A Novel Prime/Pull Therapeutic Vaccine to Prevent Recurrent Genital Herpes***" is to develop a novel and powerful T cell-based immunotherapeutic vaccine with the potential to produce a sustained clinical response to recurrent genital herpetic disease.

I am the principal investigator on this proposal and I am responsible for its conception and in coordinating the collaboration with 6 other co-investigators and collaborators (see list below).

I will supervise the data collection and analysis and I will be involved in the data reporting. I have the expertise, leadership, and motivation to successfully carry out the proposed work. I have been a principal investigator on successfully carried out several NIH R01 grant projects. I have worked on cellular and molecular immunology of infectious diseases for over 25 years, beginning as a graduate and post doc at the Pasteur Institute (France). My lab has been at the forefront of genital herpes immunology and immunotherapeutic vaccine research for 15 years and is internationally recognized as a leader in that field. I have published over 100 peer-reviewed papers, the majority in high impact journals, including *Nature Medicine, The Journal of Immunology, Mucosal Immunology*, and *The Journal of Virology.*

As an expert in genital herpes infection and mucosal immunity, I have gathered a multidisciplinary team that includes six top basic scientists in the following fields (all at UCI): (**1**) Microscopy (Dr. James V. Jester), (**2**) Mathematical modeling (Dr. Elizabeth Read), (**3**) Biostatistics (Dr. Christine McLaren), (**4**) Single-cell transcriptional profiling specialist (Dr. Melanie Oakes), (**5**) Bioinformaticist (Dr. Jenny Wu); T cell immunity and T-cell based vaccines (Dr. Rafi Ahmed).

This multidisciplinary team of leading researchers will fundamentally improve existing concepts of genital herpes infection and immunity to develop a therapeutic vaccine against recurrent herpes in the in the 6255 6255 model.

B. Positions and Honors:

Positions and Employment:

1998-1999 Post Doc, Dept. of Hematology/Bone Marrow transp., City of Hope Medical Center, CA.
1999-2000 Research Fellow: Dept. of Immunology. Beckman Research Institute, City of Hope, CA,
2001-2002 Scientist. Ophthalmology Research. Cedars-Sinai Medical Center, Los Angeles, CA.
2002-2007 Assistant Professor and Director Cellular Mol. Immunology Laboratory, UC Irvine, Irvine, CA
2007-2014 Associate Professor and Director Cellular Mol. Immunology Laboratory, UC Irvine, Irvine, CA
2014-present Full Professor & Director Cellular & Molecular Immunology Laboratory, UC Irvine, Irvine, CA

2016-present Scientist Immunologist, Sunomix Therapeutics, Inc., San Diego, CA

Other Experience and Professional Memberships:
2010-present NIH Reviewer National Institutes of Health (NIAID, NEI and NCI).
07-2010 NIH Reviewer, Member Conflict: Anterior Eye Disease (AED) Study Section [ZRG1].
02-2011 NIH Reviewer, Anterior Eye Disease (AED) Study Section.
06-2011 NIH Reviewer, NIH SBIR/STTR Grants, Small Business Diagnostic grants.
02-2012 NIH Reviewer, Strategies for the Protection of Pregnant Women (NIAID, ZAI1-BDP-M-M1).
06-2012 NIH Reviewer, Vaccines Against Microbial Diseases (VMD) Study section.
06-2013 NIH Reviewer, NIH Reviewer, Vaccines ZRG1 IMM N12.
10-2013 NIH Reviewer, Vaccine Development and Immunology (ZRG1 IM-V) study section.
11-2013 NIH Reviewer, NIAID-DAIDS-NIH-AI-2012150, Immunology Quality Assessment Program.
02-2014 NIH Reviewer, Ad-hoc reviewer NIAID. Mucosal Environment (ZAI1 RB -A (J1) Study Section.
06-2014 NIH Reviewer, Immunology Study Section (ZRG1 IMM-N12).
02-2015 NIH Reviewer, Diseases and Pathophysiology of the Visual System (DPVS) Study Section.
06-2015 NIH Reviewer, Special Emphasis Panel ZRG1 III-F 08 F, Innate Immunity and Inflammation.
06-2015 NIH Reviewer, Innate Immunity and Inflammation (III) Study Section.
07-2015 NIH Reviewer, Small Business: Non HIV Microbial Vaccines ZRG1 IMM-R(12) Study Section.
10-2015 NIH Reviewer, Immunity and Host Defense Study Section (IHD) Study Section.
02-2016 NIH Reviewer, Cellular and Molecular Immunology (CMIA) Study Section.
03-2016 NIH Reviewer, Special Emphasis Panel ZRG1-BDCN-N-55, Study Section.
05-2016 NIH Reviewer, Special Emphasis Panel ZRG1-BDCN-W-90 Study Section.
06-2016 NIH Reviewer, Cellular and Molecular Immunology (CMIA) Study Section.
02-2017 NIH Reviewer, Ocular Surface, Cornea, Anterior Segment (ZRG1-BDCN-J-81) Study Section.
02-2017 NIH Reviewer, Immunity and Host Defense Study Section (IHD) Study Section.
03-2017 NIH Reviewer, Immunology Study Section (ZRG1-IMM-C-02) Study Section.
06-2017 NIH Reviewer, Innate Immunity and Inflammation (III) Study Section.
10-2017 NIH Reviewer, Clinical Neuroimmunology and Brain Tumors Study Section (CNBT) Study Section.
10-2017 NIH Reviewer, Ocular Surface, Cornea, Anterior Segment (ZRG1-BDCN-J-81) Study Section.
11-2017 NIH Reviewer, Ocular Surface, Cornea, Anterior Segment (ZRG1-BDCN-R-03) Study Section.
03-2018 NIH Reviewer, Special Emphasis Panel ZRG1-BDCN-W-90 Study Section.
03-2018 NIH Reviewer, Ocular Surface, Cornea, Anterior Segment (ZRG1-BDCN-J-81) Study Section.
04-2018 NIH Reviewer, Member Conflict: Topics in Virology (ZRG1 IDM-W-02) Study Section.
06-2018 NIH Reviewer, Clinical Trials Study Section (ZAI1-MFH-M-S2) Study Section.
06-2018 NIH Reviewer, Cellular and Molecular Immunology Study Section (CMA) Study Section.
09-2018 NIH Reviewer, Lung Cellular, Molecular, and Immunobiology (LCMI) Study Section.
11-2018 NIH Reviewer, Sexually transmitted diseases (ZAI1-AWA-M-J1) Study Section.
01-2019 NIH Reviewer, Adjuvant Discovery/Development for Vaccines and for Autoimmune and Allergic Diseases (ZAI1-IMM-J1) Study Section.
02-2019 NIH Reviewer, Virology - A (VIRA) Study Section.

Honors:
1992-1996 Fellowship from the French Government, France
1996-1997 Fellowship from Pasteur Institute, Paris, France
1998 Award from American Society of Hematology, USA
1999 Award from American Society of Hematology, USA
2006; Award from Research to Prevent Blindness (RPB), New York, USA
2009, 2010 and 2014 Award from the Discovery Fund for Eye Research, Los Angeles, CA, USA

C. Contribution to Science:

Dr. BenMohamed's work has been highly influential in shaping the current understanding of herpes T cell-mediated immunity in both humans and 6255 models: (**1**) He developed mucosal delivery of clinically approved lipopeptide vaccines and immunotherapies to protect against herpes infection and disease. (**2**) He recently introduced a novel concept of symptomatic/asymptomatic immunology to defined the underlying mechanisms by which T cells specific to asymtomatic epitopes protect against herpes. (**3**) He discovered new immune evasion mechanisms by which HSV-1 LAT gene interferes with T cell immunity. (**4**) He

developed a novel [redacted] model of genital herpes (a model used in this proposal). (**5**) Finally, his lab has identified many HSV-1 and HSV-2 human $CD4^+$ and $CD8^+$ T cell epitopes for vaccine and immunotherapy purposes.

These five major contributions to science are detailed below:

1. Developed mucosal delivery of clinically approved vaccines and immunotherapies to protect against herpes infection and disease: Targeting of the genital mucosal immune system with subunit vaccines has failed to induce potent and durable local $CD8^+$ T cell immunity, which is crucial for protection. Dr. BenMohamed is the key developer and co-inventor of a new promising vaccine strategy that uses mucosal delivery of clinically approved lipopeptide vaccine molecules, laser adjuvant vaccine, and recently prime/pull vaccine strategy. Many researchers have now successfully tested these vaccine strategies, around the world, to protect against many infectious mucosal pathogens.

a [redacted]

b. CXCL17 Chemokine–Dependent Mobilization of CXCR8+ CD8+ Effector Memory and Tissue-Resident Memory T Cells in the Vaginal Mucosa Is Associated with Protection against Genital Herpes. Srivastava, R., Hernandez-Ruiz, M., Khan, A.A. Fouladi, M.A., Kim, G.J., Ly, V.T., Yamada, T., Lam, C., A. Sarain, S.A., Boldbaatar, U., Zlotnik, A., Bahraoui, E. & **BenMohamed L**. *The Journal of Immunology*. **2018**. 200(8):2915-2926. **PMID: 29438765**.

c. Bolstering the Number and Function of HSV-1-Specific $CD8^+$ Effector Memory T Cells and Tissue-Resident Memory T Cells in Latently Infected Trigeminal Ganglia Reduces Recurrent Ocular Herpes Infection and Disease. Khan AA, Srivastava R, Chentoufi AA, Kritzer E, Chilukuri S, Garg S, Yu DC, Vahed H, Huang L, Syed SA, Furness JN, Tran TT, Anthony NB, McLaren CE, Sidney J, Sette A, Noelle RJ, & **BenMohamed L**. *The Journal of Immunology*. **2017**. 199(1):186-203. **PMID: 28539429**.

d. A genital tract peptide epitope vaccine targeting TLR-2 efficiently induces local and systemic CD8+ T cells and protects against herpes simplex virus challenge. Zhang X, Chentoufi AA, Dasgupta G, Nesburn AB, Wu M, Zhu X, Carpenter D, Wechsler SL, You S, & **BenMohamed L.** *Mucosal Immunology*. (*Nature Publishing Group*). **2009.** 2(2):129-43. **PMID: 19129756**.

2. Discovered a novel "*asymptomatic memory $CD8^+$ T cells concept*" in herpes immunity: Generation and maintenance of high quantity and quality memory $CD8^+$ T cells determine the level of protection from viral, bacterial, and parasitic re-infections, and hence constitutes a primary goal for T cell epitope-based human vaccines and immunotherapeutics. Dr. BenMohamed recently introduced a new direction in developing T cell-based human herpes vaccines and immunotherapeutics based on the emerging new concept of "*asymptomatic memory $CD8^+$ T cells*". For this he categorized the phenotype, the function and the anatomical locations of two new major distinct sub-populations of memory symptomatic and asymptomatic HSV-specific $CD8^+$ T cells based on their protective vs. pathogenic function. Several asymptomatic HSV human epitopes have been since identified in Dr. BenMohamed's laboratory and are currently considered for T cell-based human herpes "asymptomatic" vaccine.

a. HLA-A02:01-Restricted Epitopes Identified from the Herpes Simplex Virus Tegument Protein VP11/12 Preferentially Recall Polyfunctional Effector Memory CD8+ T Cells from Seropositive Asymptomatic Individuals and Protect "Humanized" HLA-A*02:01 Transgenic Mice Against Ocular Herpes. Srivastava; R. Khan A.A., Nesburn, A.B.; Wechsler S.L. & **BenMohamed L.** *The Journal of Immunology*. **2015**. 194(5): 2232-48. **PMID: 25617474.**

b. Phenotypic and Functional Characterization of Herpes Simplex Virus Glycoprotein B Epitope-specific Effector and Memory CD8+ T Cells from Ocular Herpes Symptomatic and Asymptomatic

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

Individuals. Arif Azam Khan; Ruchi Srivastava; Doran Spencer; Daniel Fremgen; Hawa Vahed; Patricia P. Lopes; Thanh T Pham; Charlie Hewett; Jasmine Kuang; Nicolas Ong; Lei Huang; Vanessa M. Scarfone, Anthony B. Nesburn; Steven L. Wechsler & **BenMohamed L.** *The Journal of Virology*. **2015**. 89(7): 3776-92. **PMID: 25609800.**

c. [redacted]

d. Immunodominant "asymptomatic" herpes simplex virus 1 and 2 protein antigens identified by probing whole-ORFome microarrays with serum antibodies from seropositive asymptomatic versus symptomatic individuals. Dasgupta G, Chentoufi AA, Falatoonzadeh P, Chun S, Lim CH, Felgner PL, Davies DH, & **BenMohamed L**. *The Journal of Virology*. **2012**. 86(8):4358-69. **PMID: 22318137**.

3. Discovered exhaustion as a novel immune evasion mechanism of HSV-specific CD8⁺ T cells, a mechanism that is induced by herpes LAT gene expressed during herpes latency: We demonstrated, for the first time, in both [redacted] of herpes infection that most of the HSV-1-specific CD8⁺ T cells that are selectively retained in sensory ganglia, the site of latent infection, were phenotypically and functionally exhausted. In this novel immune evasion mechanisms, HSV-1 LAT gene promotes functional exhaustion (i.e., dysfunction) of HSV-specific CD8⁺ T cells resulting in virus reactivation.

a. [redacted]

b. The Herpes Simplex Virus Type 1 Latency Associated Transcript Inhibits Phenotypic and Functional Maturation of Dendritic Cells. Chentoufi, AA., Dervillez, X., Dasgupta G., Nguyen C., Kabbara, KW., Jiang X., Nesburn, A.B., Wechsler S.L. & **BenMohamed L.** *Viral Immunology*. **2012**. *(3): 204-15.* **PMID: 22512280.**

c. The Herpes Simplex Virus-1 Encoded Latency-Associated Transcript Promotes Dysfunctional Virus-Specific CD8⁺ T Cells in Latently Infected Trigeminal Ganglia: A Novel Immune Evasion Mechanism. Chentoufi, A.A., E. Kritzer, M. Tran, G. Dasgupta, R. EA., J. Xianzhi, D. Carpenter, O. Osorio, A. B. Nesburn, L. Wechsler & **BenMohamed, L**. *The Journal of Virology*. **2011**. 85(17): 9127-38. **PMID: 21715478.**

d. The herpes simplex virus type 1 latency-associated transcript can protect neuron-derived C1300 and Neuro2A cells from granzyme B-induced apoptosis and CD8 T-cell killing. Jiang X[1], Chentoufi AA, Hsiang C, Carpenter D, Osorio N, **BenMohamed L**, Fraser NW, Jones C, Wechsler SL. *The Journal of Virology*. **2011**. 85(5): 2325-32. **PMID: 21177822.**

4. Developed "humanized" [redacted] of herpes infection and disease: The choice of the right [redacted] model that reliably reflects recurrent herpes infection and disease, as occurs in humans, is crucial in determining the underlying immune mechanisms that control recurrent herpes. We recently developed an a [redacted] for UV-B induced recurrent herpetic corneal disease.

a. [redacted]

b. Prior Corneal Scarification and Injection of Immune Serum are not Required Before Ocular HSV-1 Infection for UV-B Induced Virus Reactivation and Recurrent Herpetic Corneal Disease in Latently Infected Mice. **BenMohamed L**.; Osorio N.; Khan A.A.; Srivastava R.; Huang L.; Krochmal J.J. Garcia, J.M. Simpson J.L.; and Wechsler. S.L. *Current Eye Research*. **2015**. *41(6):747-56.* **PMID: 26398722.**

c. [redacted]

d.

5. Leader in mapping of human CD4⁺ and CD8⁺ T cell epitopes from HSV-1 protein antigens for ocular herpes vaccine and immunotherapy purposes: Dr. BenMohamed's efforts in last 2 decades had let to identification of several CD4⁺ and CD8⁺ T cell epitopes from many herpes glycoprotein and tegument proteins that are currently being considered fro clinical herpes vaccine trials.

a. [redacted]

b.

c. HLA-A*0201-restricted CD8+ cytotoxic T lymphocyte epitopes identified from herpes simplex virus glycoprotein D. Chentoufi AA, Zhang X, Lamberth K, Dasgupta G, Bettahi I, Nguyen A, Wu M, Zhu X, Mohebbi A, Buus S, Wechsler SL, Nesburn AB. & **BenMohamed L**. *The Journal of Immunology*. **2008**. 180(1): 426-437. **PMID: 18097044**.

d. Asymptomatic human CD4+ cytotoxic T-cell epitopes identified from herpes simplex virus glycoprotein B. Chentoufi AA, Binder NR, Berka N, Durand G, Nguyen A, Bettahi I, Maillère B., & **BenMohamed, L**. *The Journal of Virology*. **2008**. 82(23): 11792-802. **PMID: 18799581.**

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/pubmed/?term=Benmohamed+l

D. Ongoing Research Support:

1. [redacted]

2. R01 EY026103-01A1. (**BenMohamed, PI**). Mechanisms of CD8⁺ T Cell Dynamics in Recurrent Ocular Herpetic Disease. NIH/NEI Period: **04/01/16 - 03/31/2020.**

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Burkhard, Peter

eRA COMMONS USER NAME (credential, e.g., agency login): PETERBURKHARD

POSITION TITLE: CEO

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Biozentrum, University of Basel, Basel, Switzerland	Diploma	1992	Biochemistry
Sandoz Pharma AG, Basel, Switzerland	PhD	1995	Biophysics
Biozentrum, University of Basel, Basel, Switzerland	Postdoc	1998	Structural Biology
Biozentrum, University of Basel, Basel, Switzerland	Habilitation	2001	Structural Biology

A. Personal Statement

My experience and qualifications make me particularly well-suited for the role as co-PI in this HSV nanoparticle vaccine project. The nanoparticles have been invented by me about fifteen years ago. Ever since I have continuously and with great enthusiasm further developed the nanoparticles to make them suitable as a platform for vaccine design. I have analyzed their biophysical and immunological properties in great detail resulting in the production of five vaccine prototypes for four different infectious diseases that all are almost completely protective in 6255 challenge models. This work resulted in five different patents / patent applications of SAPNs for vaccine design (US8575110, US8546337, EP2766386A1, EP2766386A1, EP17157687.9). I have also advanced our most developed malaria SAPN vaccine up to the stage of clinical trials phase I/IIa, which is planned to be finished in summer (2018). Furthermore, I have founded the company Alpha-O Peptides more than a decade ago. Developing vaccines is the core business of this company. For all those reasons, I think that I am perfectly qualified to be the lead PI at Alpha-O Peptides in this HSV vaccine project. I strive to develop the HSV vaccine to possibly also bring it into clinical trials as quickly as possible. My personal background covering everything from nano-biotechnology to immunology and including all aspects that are important for vaccine design makes me perfectly suited to direct the research of this proposal.

I will collaborate with the principal investigator, Dr. BenMohamed from the UC Irvine on this proposal that is developing the prime pull/vaccine against genital herpes. The scope of work of this R01 grant entitled "***A Novel Prime/Pull Therapeutic Vaccine to Prevent Recurrent Genital Herpes***" is to develop a Self-Assembling Protein Nanoparticles (SAPNs) combined with T-cell attracting chemokines against recurrent genital herpes in the 6255 model. Self-Assembling Protein Nanoparticles (SAPNs) will be provide UC Irvine during the first 2-years of this project. The Vaccine will then be tested in the 6255 model of recurrent herpes at UC Irvine.

The following are my most relevant patents / patent applications.

a) US8575110 (2004). "Peptidic Nanoparticles as Drug Delivery and Antigen Display Systems" **P. Burkhard**

b) US 8546337 (2008). “Self-assembling peptide nanoparticles useful as vaccines” **P. Burkhard**
c) WO2015104352 (2014). “Flagellin-containing protein nanoparticles as a vaccine platform” by S.K. Raman, S.M. Paulillo, M. Piazza, C. Kulangara, C. Mittelholzer, and **P. Burkhard**
d) EP17157687.9 (2017). “Self-assembling protein nanoparticles encapsulating immunostimulatory nucleid acids” by S.K. Raman, S.M. Paulillo, C. Kulangara, M. Piazza and **P. Burkhard**

B. Positions and Honors

- 1995 - 1998 Postdoctoral Position at the Biozentrum, University of Basel, CH
- 1998 - 2004 Group leader at the Biozentrum, University of Basel, CH
- 2001 Habilitation, University of Basel, CH
- 2003 Founder of Alpha-O Peptides, AG, Riehen, CH
- 2004 - 2013 Associate Professor, University of Connecticut, CT, USA
- 2013 - 2015 Full Professor, University of Connecticut, CT, USA
- 2015 - 2016 Research Professor, University of Connecticut, CT, USA
- 2003 - CEO of Alpha-O Peptides, AG, Riehen, CH

- 2005 Senior Founding Member of the American Academy of Nanomedicine
- 2006 Fellow of the American Academy of Nanomedicine
- 2010 Tenured faculty position at the University of Connecticut
- 2011 - Editor of the Journal of Nanobiotechnology
- 2011 Director's Award for Faculty Excellence, Polymer Program, University of Connecticut
- 2012 - Editor of Current Bionanotechnology

C. Contribution to Science

1. Protein Structural Analysis for Structure Based Design

DOPA decarboxylase (DDC) is responsible for the synthesis of the key neurotransmitters dopamine and serotonin via decarboxylation of L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan, respectively. DDC has been implicated in a number of clinic disorders, including Parkinson’s disease and hypertension. Peripheral inhibitors of DDC are currently used to treat these diseases. We have solved the X-ray crystal structures of ligand-free DDC and its complex with the anti-Parkinson drug carbiDOPA. The inhibitor is bound to the enzyme by forming a hydrazone linkage with the cofactor, and its catechol ring is deeply buried in the active site cleft. These structures provide the molecular basis for the development of new inhibitors of DDC with better pharmacological characteristics. P. Burkhard et al. (2001) Nature Struct Biol, 8 (11), 963 – 967).

Publication of these DDC structures prompted Rebecca Craven to write the following comments in the Highlights section in Nature Reviews Neuroscience (2002) 2 (12), 855: *The treatment of patients with Parkinson’s disease could be greatly improved by the design of more effective inhibitors of this enzyme. This prospect seems increasingly likely, as Burkhard et al. report the crystal structures of ligand-free DCC, and its complex with carbiDOPA. Importantly, on the basis of these structures, the authors were able to suggest ways in which the binding of inhibitors of DCC might be improved. The use of more-potent inhibitors of DCC would allow smaller amounts L-DOPA to be used in alleviating the symptoms of Parkinson’s disease; the crystal structures reported by Burkhard et al. offer a way forward in the design of such treatments.*

e) **Burkhard, P.**, Dominici, P., Borri-Voltattorni, C., Jansonius, J.N., and Malashkevich, V.N. Structural insight into Parkinson's disease treatment gained from drug-inhibited DOPA decarboxylase. *Nature Struct Biol*. 2001 Nov;8(11):963-967. PMID: 11685243

f) Meier, M., Janosik, M., Kery, V., Kraus, J. and **Burkhard, P**. Structure of human cystathionine beta-synthase: a unique pyridoxal 5'-phosphate-dependent heme protein. *EMBO J*. 2001 Aug 1;20(15):3910-3916. PMCID: PMC149156

g) Stetefeld, J., Jenny, M., and **Burkhard, P**. Intersubunit signaling in glutamate-1-semialdehyde-aminomutase. *Proc Natl Acad Sci U S A*. 2006 Sep 12;103(37):13688-13693. PMCID: PMC1564225

h) **Burkhard, P**., Rao, G.S., Hohenester, E., Schnackerz, K.D., Cook, P.F. & Jansonius, J.N. Three-dimensional Structure of *O*-acetylserine Sulfhydrylase from Salmonella typhimurium. *J Mol Biol*. 1998;283(1):121-133. PMID: 9761678

2. Structural Design and Analysis of Coiled-coil Proteins

The parallel two-stranded α-helical coiled coil is the most frequently encountered subunit-oligomerization motif in proteins. The simplicity and regularity of this motif have made it an attractive system to explore some of the fundamental principles of protein folding and stability and to test the principles of de novo design. We have solved the X-ray crystal structure of the 18-heptad-repeat α-helical coiled-coil domain of the actin-bundling protein cortexillin I from Dictyostelium discoideum and shown that it is a tightly packed parallel two-stranded α-helical coiled coil. It harbors a distinct 14-residue sequence motif that is essential for coiled-coil formation, and is a prerequisite for the assembly of cortexillin I. The knowledge gained from the structure can be used in the de novo design of α-helical coiled coils for applications such as two-stage drug targeting and delivery systems, and in the design of coiled coils as templates for combinatorial helical libraries in drug discovery and as synthetic carrier molecules. (P. Burkhard et al. (2000). *Structure*, **8**, 223-230.)

Presentation of this structure at the American Crystallographic Association Annual Meeting 1999 in Washington triggered the following Editorial Reprise in *Nature Struct. Biol*., 5, (1998), 762 by Guy Riddihough. *Perhaps the most apposite example was provided by P. Burkhard who reported on the structure determination of the 190 Å long α-helical, two-stranded, right-handed coiled-coil rod domain from cortexillin I. This is the longest structure of a coiled coil reported to date, soundly beating the 39-residue long cFos-cJun bZIP leucine zipper. The rod domain includes a 13-residue 'trigger site' that has been shown to be necessary for coiled coil assembly and, indeed, has been characterized as an autonomous folding unit, suggesting that this is a general feature of coiled coil assembly.*

a) Strelkov, S., Herrmann, H., Geisler, N., Zimbelmann, R., Aebi, U. and **Burkhard, P**. Conserved segments 1A and 2B of the intermediate filament dimer: their atomic structures and role in filament assembly. *EMBO J*. 2002 Mar 15;21(6):1255-1266. PMCID: PMC125921

b) Strelkov, S.V., and **Burkhard, P**. Analysis of alpha-helical coiled coils with the program TWISTER reveals a structural mechanism for stutter compensation. *J Struct Biol*. 2002 Jan-Feb;137(1-2):54-64. PMID: 12064933

c) **Burkhard, P**., Kammerer, R.A., Steinmetz, M.O., Bourenkov, G.P. and Aebi, U. The coiled-coil trigger site of the rod domain of cortexillin I unveils a distinct network of inter- and intra-helical salt-bridges. *Structure*. 2000 Mar 15;8(3):223-230. PMID: 10745004

d) **Burkhard, P**., Meier, M. and Lustig, A. Design of a minimal protein oligomerization domain by a structural approach. *Protein Science*. 2000 Dec;9(12):2294-2301. PMCID: PMC2144530

3. Structural Design of Self-Assembling Protein Nanoparticles (SAPNs)

Artificial particulate systems such as polymeric beads and liposomes are being applied in drug delivery, drug targeting, antigen display, vaccination, and other technologies. We have used computer modeling to design a novel type of self-assembling protein nanoparticles (SAPNs) composed of proteins as building blocks. We describe the structure-based design of a novel type of nanoparticles with regular polyhedral symmetry and a diameter of about 16 nm, which self-assembles from single protein chains. Each protein chain is composed of two coiled coil oligomerization domains with different oligomerization states joined by a short linker segment. In aqueous solution the proteins form nanoparticles of about 20 nm diameter. Such protein nanoparticles are ideally suited for medical applications such as drug targeting and drug delivery systems, as imaging devices, or they may be used for repetitive antigen display.

a) Raman, S.K., Machaidze, G., Lustig, A., Aebi, U. and **Burkhard, P**. Structure-based design of peptides that self-assemble into regular polyhedral nanoparticles. *Nanomedicine*. 2006 Jun;2(2):95-102. PMID: 17292121

b) Pimentel T.A., Yan Z, Jeffers S.A., Holmes K.V., Hodges R.S. and **Burkhard P**. Peptide nanoparticles as novel immunogens: design and analysis of a prototypic severe acute respiratory syndrome vaccine. *Chemical Biology and Drug Design*. 2009 Jan;73(1):53-61. PMCID: PMC2756483

c) Yang Y., Ringler P., Mueller S.A. and **Burkhard P**. Optimizing the refolding conditions of self-assembling polypeptide nanoparticles that serve as repetitive antigen display systems. *J Struct Biol.* 2012 Jan;177(1):168-176. PMID: 22115997

d) Indelicato G., Wahome N., Ringler P., Müller S.A., Nieh M., **Burkhard P** and Twarock R. Principles Governing the Self-Assembly of Coiled-Coil Protein Nanoparticles. *Biophys J*. 2016 Feb 2;110(3):646-660. PMCID: PMC4744166

4. Vaccine design using SAPNs

Using the SAPNs as a platform for vaccine design, I have demonstrated that the SAPNs can be used as a general platform for vaccine design. I have five different patents / patent applications dealing with the use of SAPNs for vaccine design (US8575110, US8546337, EP2766386A1, EP2766386A1, EP17157687.9). In the research labs of Alpha-O Peptides we have engineered five vaccine prototypes for four different infectious diseases that all are almost completely protective in 6255 challenge models. The clinical trials phase I/IIa of the most advanced vaccine (malaria) is currently planned to be finished next summer (2018). The five prototypes are: Malaria vaccine, HPV vaccine (L2-based), universal flu vaccine (M2e- and Helix C-based), seasonal flu vaccine (HA-based), toxoplasmosis vaccine. All of those prototypes are bacterially expressed, most of them are composed of one single protein chain. So, they can be produced very cheaply and rapidly. These five prototype vaccines show that the SAPN technology is indeed a platform technology that can be quickly adapted to pretty much any infectious disease (Ebola, Zika, Chikungunya, etc.). Furthermore, the SAPN technology can be used to engineer therapeutic vaccines for cancer, Alzheimer, addictions, obesity and many more.

a) Karch CP, Doll TAPF, Paulillo SM, Nebie I, Lanar DE, Corradin G, **Burkhard P** (2017). The Use of a P. falciparum Specific Coiled-coil Domain to Construct a Self-Assembling Protein Nanoparticle Vaccine to Prevent Malaria. *J. Nanobiotechnology*, 15 (1), 62 doi: 10.1186/s12951-017-0295-0

b) Kaba SA, Karch CP, Seth L, Ferlez KMB, Storme CK, Pesavento DM, Laughlin PY, Bergmann-Leitner ES, **Burkhard P**, Lanar DE (2018). Self-assembling protein nanoparticles with built-in flagellin domains increases protective efficacy of a Plasmodium falciparum based vaccine. *Vaccine* 36(6), 906-914. doi: 10.1016/j.vaccine.2017.12.001.

c) El-Bissati K, Zhou Y, Paulillo SM, Raman SK, Karch CP, Roberts CW, Lanar DE, Reed S, Fox C, Carter D, Alexander J, Sette A, Sidney J, Lorenzi H, Begeman IJ, **Burkhard P**, McLeod R (2017). Protein nanovaccine confers robust immunity against Toxoplasma. *Nature PJ Vaccines*, 2, doi:10.1038/s41541-017-0024-6.

d) Karch CP, Li J, Kulangara C, Paulillo SM, Raman SK, Emadi S, Tan A, Helal ZH, Fan Q, Khan MI, **Burkhard P**. Vaccination with self-adjuvanted protein nanoparticles provides protection against lethal influenza challenge. *Nanomedicine*. 2017 Jan;13(1):241-251. doi: 10.1016/j.nano.2016.08.030.

Complete List of Published Work in NCBI

https://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/42368162/

D. Research Support

Ongoing Research Support

None.

Completed Research Support

Development of novel IBV-nanoparticle based vaccine, its immunogenicity and protection studies in chickens

Role Co-PI Duration: 05/15 - 04/18 Funding agency: USDA-NIFA

Overall goal: To design protein nanoparticles as subunit vaccine against IBV.

Responsibilities: To direct the research in the Burkhard lab at UConn and coordinate with the research group of the PI M. Khan at the University of Connecticut.

GMP Production and Clinical Trial of a Self-Assembling Protein Nanoparticle and Toll-Like Receptor Liposomal MPL Adjuvanted Malaria Vaccine

Role Co-PI Duration: 07/15 - 06/17 Funding agency: CDRMP
Overall goal: To test a malaria vaccine based on self-assembling protein nanoparticles in clinical trials.
Responsibilities: To consult on the bio-production and vaccination protocols for the self-assembling protein nanoparticles developed at Alpha-O Peptides AG.

Malaria Vaccine Based on Self-Assembling Polypeptide Nanoparticles (SAPN)

Role PI Duration: 08/09 - 07/13 Funding agency: NIH-NIAID
Overall goal: This R01 proposal has the goal to design peptide nanoparticles as subunit vaccine against malaria.
Responsibilities: To direct the research at UConn and coordinate with the research group at WRAIR.

Atomic structure and assembly of Intermediate Filaments

Role PI Duration: 05/11 - 12/16 Funding agency: NIH-NIGMS
Overall goal: The goal of this PPG-project is to investigate the structural and biophysical properties of the intermediate filament protein vimentin
Responsibilities: To direct the research in the Burkhard lab at UConn and coordinate with the research group at Harvard, Northwestern and UPenn.

A peptide nanoparticle nicotine vaccine

Role PI Duration: 09/11 - 12/16 Funding agency: NIH-NIDA
Overall goal: This DP1 award aims at the development of a peptide nanoparticle nicotine vaccine and advance it through clinical trials phase I.
Responsibilities: To direct the whole project at UConn (protein design) at Alpha-O Peptides in Riehen (biophysical analysis), Switzerland and the Kantonsspital St. Gallen, Switzerland (clinical trials).

Peptide Nanoparticles as Novel Immunogens: Design and Analysis of Avian Influenza Vaccine

Role PI Duration: 12/11 - 11/16 Funding agency: USDA-NIFA
Overall goal: To design peptide nanoparticles as subunit vaccine against malaria.
Responsibilities: To direct the research in the Burkhard lab at UConn and coordinate with the research group of Dr. Khan (UConn - PI) and Gelb (University of Maryland).

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

NAME: McLaren, Christine E.

eRA COMMONS USER NAME (credential, e.g., agency login): cmclaren

POSITION TITLE: Professor of Biostatistics

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
California State University, San Jose, CA	BS (Honors)	06/69	Mathematics
Stanford University, Palo Alto, CA	MA	06/70	Mathematics Education
Case Western Reserve University, Cleveland, OH	MS	06/76	Mathematical Statistics
Case Western Reserve University, Cleveland, OH	PhD	06/83	Biostatistics

A. Personal Statement

I am Professor and Vice Chair, Department of Epidemiology and I am Interim Chair of the Biostatistics Shared Resource, Chao Family Comprehensive Cancer Center (CFCCC). I have over 25 years of experience in the design, conduct, and statistical analysis of research studies. I have focused on statistical modeling research that provides insight into biological processes distinguishing between health and disease. In 1993, I was elected a Fellow of the American Statistical Association, in part for "innovative research in biology and medicine".

I have a longstanding and successful working relationship Dr. BenMohamed. For this project, I will provide analysis of $CD4^+$ and $CD8^+$ T cell function and exhaustion to genital herpes simplex in the 6255 model following prime/pull vaccine. I will provide detailed descriptive and analytic reports and help analyze the statistics of the *in vitro*, *in situ, ex vivo*, and *in vivo* results. I will also analyze the statistics comparing the contribution of the peripheral epithelial T cell immunity (at the VMC) epithelium vs. central neuronal T cell immunity (at the DRG) in protection against recurrent genital herpes in 6255 This will include: (1) Statistical analysis to capture the $CD4^+$ and $CD8^+$ T_{RM} cell dynamics of the containment within HSV-2 infected DRG and VMC. This includes statistical analysis of HSV-2 reactivation from DRG based on observed patterns of single neuron loads and $CD4^+$ and $CD8^+$ T cell infiltration and HSV-2 shedding rate from VMC; and (2) Statistical analysis to characterize the duration of protection, and the protective mechanisms induced by the prime/pull vaccine in the 6255 model. We expect the statistical analysis, to help determine relative contribution of the peripheral epithelial T cell immunity epithelium vs. central neuronal T cell immunity in protection against recurrent genital herpes.

B. Positions and Honors

Positions and Employment

1976-79 Research Biostatistician, Department of Biometry, Case Western Reserve University
1979-80 Research Officer, Department of Haemotology, Welsh National School of Medicine
1980-83 Research Biostatistician, Department of Biometry, Case Western Reserve University
1983-84 Senior Instructor, Department of Biometry and Department of Medicine (Cleveland Metropolitan General Hospital), Case Western Reserve University
1984-86 Assistant Professor, Department of Biometry and Department of Medicine (Cleveland Metropolitan General Hospital), Case Western Reserve University
1986-87 Assistant Professor, Department of Mathematics, Minnesota State University Moorhead
1987-92 Associate Professor, Department of Mathematics, Minnesota State University Moorhead
1992-98 Professor, Department of Mathematics, Minnesota State University Moorhead
1998-present Professor of Medicine (Epidemiology) and Director of Biostatistics (Chao Family Comprehensive Cancer Center), University of California, Irvine
2008-present Vice Chair for Academic Affairs, Department of Epidemiology, University of California, Irvine

Other Experience and Professional Memberships

1984-2002 International Committee for Standardization in Hematology (Cytometry), Statistical Consultant

1990, 1999 National Science Foundation, Division of Mathematical Sciences, Grant Review Panel
1994, 2000-04 National Institutes of Health, Statistical Reviewer, Hematology Study Section, CSR
2001-2004 Veterans Health Administration, Member, Epidemiology Merit Review Subcommittee
2005-2006 NIH, Ad-hoc Reviewer, NCI Clinical Oncology Study Section
2006 NIH, NCI Initial Review, NCI-A RTRB-H (L1), Subcommittee A – Cancer Centers
2007 NIH Ad-hoc Reviewer, Subcommittee 1-Career Development
2007-2011 NIH, Member, NCI Clinical Oncology (CONC) Study Section
2012 NCI, Reviewer, SPORE in Breast, Endometrial, and Skin Cancers, ZCA1 RPRB-0 M1 P
2013 NCI Oncology 2 - Translational Clinical Integrated Review Group
2014 NCI, Reviewer, P01 Special Emphasis Panel III, ZCA1 RPRB-0 (J1)
2015 NCI, Reviewer, Special Emphasis Panel, ZCA1 PCRB-C (M1) R
2016 NCI Specialized Programs of Research Excellence (SPORE) Review Group
2016 NCI, Chair of Omnibus SEP16 R03 & R21 Review Group, 2016/05 ZCA1 PCRB-C (M1) S
2016 NCI Specialized Programs of Research Excellence (SPORE) Review Group
2018 NCI Specialized Programs of Research Excellence (SPORE) Review Groups

Honors

1983-84 American Heart Association Research Fellowship
1985 Visiting Scientific Officer, University of Wales College of Medicine, United Kingdom
1986-present Fellow, Royal Statistical Society
1991 Senior Honorary Research Fellowship, University of Glasgow, United Kingdom
1993 Phi Kappa Phi Honor Society
1993-present Fellow, American Statistical Association
1994-1995 Raybould Visiting Fellowship, Dept. of Mathematics, Univ. of Queensland, Brisbane, Australia
1995 Senior International Fellowship awarded by the NIH Fogarty International Center
1996 University Dean's Council Nominee, 1997 US Professors of the Year Program, Carnegie Foundation for the Advancement of Teaching
2004 American Statistical Association Service Award, Council of Chapters
2013 Clinical and Translational Science (ICTS) Interdisciplinary Team Science Award, Athena Breast Health Network Program, University of California, Irvine
2014 "Best of ASH" award, 56th meeting of the American Society of Hematology, Dec. 5-9, 2015
2017 Elected Member, International Statistical Institute
2017 Albert Nelson Marquis Lifetime Achievement Award

C. Contributions to Science

1. **Collaborative Research in Cancer**. My collaborative efforts in optical and magnetic imaging are illustrated by my participation as a co-investigator and lead biostatistician for multiple grants. I have co-authored publications resulting from studies of dynamic contrast-enhanced magnetic resonance imaging as a clinical imaging modality for the detection, diagnosis, and treatment of breast lesions. As an example, I supervised statistical modeling using generalized estimating equations (GEE) models that incorporated therapy response, treatment regimen, measurement day, and interaction terms to assess the outcomes of oxyhemoglobin, deoxyhemoglobin, water, and lipid. The results showed that functional hemodynamic and metabolic information acquired using a noninvasive optical imaging method on the first day after neoadjuvant chemotherapy treatment can discriminate nonresponding from responding patients. As Director of the Data Coordinating Center for NIH/NCI grant R01 CA88078-01 (F.L. Meyskens, P.I.), I provided analyses and interpretation of data from the landmark study that demonstrated that recurrent adenomatous polyps can be markedly reduced by a combination of low oral doses of difluoromethylornithine and sulindac and with few side effects.
   a. **McLaren CE**, Fujikawa-Brooks S, Chen W-P, Gillen DL, Pelot D. Gerner EW, Meyskens FL. Longitudinal assessment of air conduction audiograms in a phase III clinical trial of DFMO and sulindac for prevention of sporadic colorectal adenomas. Cancer Prev Res 1:514-521, 2008. PMCID:PMC2702261.
   b. **McLaren CE**, Chen W-P, Nie K, Su M-Y. Prediction of malignant breast lesions from MRI features: a comparison of artificial neural network and logistic regression techniques. Acad Radiol 16(7):842-51, 2009. PMCID: PMC2832583

c. O'Sullivan TD, Leproux A, Chen JH, Bahri S, Matlock A, Roblyer D, **McLaren CE**, Chen WP, Cerussi AE, Su MY, Tromberg BJ. Optical imaging correlates with magnetic resonance imaging breast density and reveals composition changes during neoadjuvant chemotherapy. Breast Cancer Res 15(1):R14, 2013. PMCID: PMC3672664.

2. **Hemochromatosis and Iron Overload**. Hemochromatosis is a hereditary disease in which affected persons suffer excessive dietary iron absorption and may lead to complications such as liver cirrhosis, hepatocellular carcinoma, heart failure, diabetes, arthritis, and impotence. I have 24 years of experience working on hematological studies and have published methodological and applied papers related to hemochromotosis, iron overload, and measures of iron status. As Principal Investigator of a Field Center for the Hemochromatosis and Iron Overload Screening (HEIRS) Study, I was lead author on the initial paper describing the overall study design. I supervised enrollment of 20,400 participants at the University of California, Irvine. Based on data from 99,711 participants, we found that the C282Y (substitution of tyrosine for cysteine at amino acid 282) mutation of the *HFE* gene is most common in whites and is accompanied by elevations on iron measures. As a co-investigator for The Melbourne Collaborative Cohort Study, I was co-author of papers describing results from the prospective cohort in which participants born in Australia, New Zealand, the United Kingdom, or Ireland (n=28,509) were genotyped for the *HFE* C282Y variant. Iron-overload-related disease developed in a substantial proportion of C282Y homozygous men. *HFE* C282Y homozygotes have twice the risk of colorectal and breast cancer compared with those individuals without the C282Y variant.
   a. **McLaren CE**, Barton JC, Adams PC, Harris EL, Acton RT, Press N, Reboussin DM, McLaren GD, Sholinsky P, Walker AP, Gordeuk VR, Leiendecker-Foster C, Dawkins FW, Eckfeldt JH, Mellen BG, Speechley M, Thomson E for the Hemochromatosis and Iron Overload Study Research Investigators. Hemochromatosis and iron overload screening (HEIRS) Study Design for an Evaluation of 100,000 primary care-based adults. The Am J Med Sci 325:53-62, 2003. PMID: 12589228..
   b. **McLaren CE**, Gordeuk VR, Chen WP, Barton JC, Acton RT, Speechley M, Castro O, Adams PC, Snively BM, Harris EL, Reboussin DM, McLachlan GJ, Bean R. Bivariate mixture modeling of transferrin saturation and serum ferritin concentration in Asians, African Americans, Hispanics, and Whites in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. Trans Res 151(2):97-109, 2008. PMCID: PMC3785302.
   c. Osborne NJ, Gurrin LC, Allen KJ, Constantine CC, Delatycki MB, **McLaren CE**, Gertig DM, Anderson GJ, Olynyk JK, Powell LW, Hopper JL, Giles GG, English DR. HFE C282Y homozygotes are at increased risk of breast and colorectal cancer. Hepatology 51(4):1311-8, 2009. PMCID: PMC3815603.

3. **Genetic Components of Iron Status**. As PI **OF** NIH grant R01-HL083328-01A1, “Iron Status: A Pathway Analysis in Multiple Ethnicities”, I led a multi-center project to study the heritability of serum iron measures, determine single nucleotide polymorphisms (SNPs) and haplotypes in key genes involved in systemic iron metabolism pathways, identify potential cases of iron deficiency and controls, and study the association between the presence of iron deficiency and haplotypes in the selected candidate genes. Heritability is the proportion of observed variation in a trait among individuals in a population that is attributable to hereditary factors. Participants (N=942) were 77% Caucasians, 10% Asians, 8% Hispanics, and 5% other race/ethnicities. We found that serum iron measures have significant heritability components, after excluding known genetic and nongenetic sources of variation**.** Subsequently, we performed a genome-wide association study (GWAS) using DNA collected from participants in the HEIRS Study to identify new genomic locations associated with iron deficiency. Replication analyses were performed in a sample of veterans screened at a US Veterans Affairs (VA) medical center. The joint analysis of the HEIRS and VA samples revealed strong associations between rs2698530 on chr. 2p14 and iron status outcomes, confirming a previously-described *TF* polymorphism and implicating one potential new locus as a target for gene identification**.** A follow-up study of white, African-American, Hispanic, and Asian HEIRS participants analyzed the association between SNPs and eight iron-related outcomes. Three chromosomal regions showed association across multiple populations, including SNPs in the *TF* and *TMPRSS6* genes, and on chromosome 18q21. A novel SNP rs1421312 in *TMPRSS6* was associated with serum iron in whites ($P=3.7x10^{-6}$) and replicated in African Americans (P = 0.0012). Our results confirmed known associations with iron measures and gave unique evidence of their role in different ethnicities, suggesting origins in a common founder. I am currently the PI of a separate multi-site NIH grant 1R24 DK099846-01A1, “Genetic Modifiers of Iron Status in Hemochromatosis *HFE* C282Y Homozygotes”. We hypothesized that variants of genes other than *HFE* and those previously associated with hemochromatosis and iron overload

phenotypes are involved in the regulation of iron metabolism and modulate expression of iron overload in *HFE* C282Y homozygotes.

a. **McLaren CE**, Barton JC, Eckfeldt JH, McLaren GD, Acton RT, Adams PC, Henkin LF, Gordeuk VR, Vulpe CD, Harris EL, Harrison BW, Reiss JA, Snively BM. Heritability of Serum Iron Measures in the Hemochromatosis and Iron Overload Screening (HEIRS) Family Study, Am J Hematol 85(2):101-5, 2010. PMCID: PMC3816512.
b. **McLaren CE**, Garner CP, Constantine CC, McLachlan S, Vulpe CD, Snivelely BM, Gordeuk VR, Nickerson DA, Cook JD, Leiendecker-Foster C, Beckman KB, Eckfeldt JH, Barcellos LF, Murray JA, Adams PC, Acton RT, Killeen AA, McLaren GD. James D. Genome-wide association study identifies genetic loci associated with iron deficiency. PLoS ONE 6(3):e17390, 2011. PMCID: PMC3069025.
c. **McLaren CE**, McLachlan S, Garner CP, Vulpe CD, Gordeuk VR, Eckfeldt JH, Adams PC, Acton RT, Murray JA, Leiendecker-Foster C, Snively BM, Barcellos LF, Cook JD, McLaren GD. Associations between single nucleotide polymorphisms in iron-related genes and iron status in multiethnic populations. PLoS One 7(6):e38339, 2012. PMCID: PMC3382217.
d. **McLaren CE**, Emond MJ, Subramaniam N, Phatak PD, Barton JC, Adams PC, Goh JB, McDonald CJ, Powell LW, Gurrin LC, Allen KJ, Nickerson DA, Louie T, Ramm, GA, Anderson GJ, McLaren GD. Exome sequencing in HFE C282Y homozygous men with extreme phenotypes identifies a GNPAT variant associated with severe iron overload. Hepatology 62(2):429-439, 2015. PMCID: PMC450823.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/christine.mclaren.1/bibliography/44197942/public/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1 R21 HL145232-01 CE McLaren (PI) 09/15/18-08/31/20
NIH/NHLBI
"Modulation of Iron Overload by Hepcidin and Erythroferrone"
This research will conduct a collaborative study to characterize the utility of serum hepcidin concentration and erythroferrone in identifying hemochromatosis patients who are at greatest risk of developing severe iron overload.
Role: PI

5 R24 DK 099846-03 CE McLaren/GD McLaren (PIs) 09/01/14-06/31/19
NIH/NIDDK
"Genetic Modifiers of Iron Status in Hemochromatosis HFE C282Y Homozygotes"
This research is to conduct a collaborative study that will answer the question, "What role do genetic modifiers play in determining iron accumulation in persons homozygous for the HFE C282Y genotype?"
Role: PI

1R21 CA208938 (PI: Su, M-Y) 08/01/17 – 7/31/19
NIH/NCI
Mammographic Density and Metabolic Genotyping for Predicting Cancer Prognosis"
This project will investigate the role of quantitative mammographic density (MD) and cytochrome P450 CYP2D6 metabolic genotyping in predicting the prognosis of breast cancer patients with hormonal receptor positive breast cancer receiving Tamoxifen treatment.
Role: Co-Investigator

2 P30 CA 062203-20, Van Etten, R. (PI) 09/11/97-01/31/21
NIH/NCI
"Cancer Center Support Grant"
The Cancer Center Support Grant provides support for administration and infrastructure for the UC Irvine Chao Family Comprehensive Cancer Center. Dr. McLaren is co-Leader of the Program in Cancer Control and Interim Interim Director of the Biostatistics Shared Resource.
Role: Co-Investigator

5 DP7 OD 020321-04 Fruman (PI) 09/18/14-08/31/19
NIH/OD
"UCI-GPS: UC Irvine Graduate Professional Success"
This is a campus-wide effort at UC Irvine to broaden the training of biomedical PhD students and postdoctoral fellows and to encourage students and postdoctoral fellows to prepare for a variety of career options.
Role: Co-Investigator

5 R01 EY 019896-07 BenMohamed (PI) 09/01/10-03/31/20
NIH/NEI
Therapeutic Ocular HSV Vaccine in HLA Transgenic Rabbits
The ultimate objective of the proposed mechanistic and translational research, which uses a unique HLA transgenic rabbit model of HSV-1 spontaneous reactivation, as determined by shedding of reactivated virus in tears, is to understand the interaction between CD8+ T cells and HSV-1.
Role: Co-investigator

5 R01 EY 026103-02 BenMohamed (PI) 08/01/16-7/31/20
NIH/EYI
Mechanisms of CD8+ T Cell Dynamics in Recurrent Ocular Herpetic Disease
This is mechanistic and translational preclinical research of recurrent ocular herpes disease, caused by HSV-1 infection, designed to develop a clinical T-cell based immunotherapy against recurrent ocular herpes.
Role: Co-investigator

5 R01 CA 127927-10 Su (PI) 04/01/07-07/31/19
NIH/NC
"Predicting Neoadjuvant Chemo Response/Prognosis Using Imaging Biomarkers"
This project will investigate the role of imaging markers measured by MRI and breast-scintigraphic imaging for predicting the response of breast cancer patients undergoing neoadjuvant chemotherapy (NAC).
Role: Co-investigator

Completed Research Support (selected)
5 R01EY024618-03 BenMohamed (PI) 09/03/14-08/31/18
NIH/NEI
"Blockade of T-cell Co-Inhibitory Pathways and Immunotherapy to Prevent Ocular Herpes"
The goals of this translational project are to understand the T-cell co-inhibitory dependent mechanisms used by HSV-1 to evade CD8+ T cell immunosurveillance and to devise a novel T cell-based immunotherapy.
Role: Co-investigator

1R21 CA166839-01A1 (M. Lilly and Z. Zi, MPI) 09/01/13-08/31/15
Phase 1 bioassay-guided Trial of Lycopene and Docetaxel for Prostate Cancer
This research will perform a Phase I trial of lycopene in combination with docetaxel as first-line chemo-therapy for patients with castration-resistant prostate cancer.
Role: Co-investigator

1R21 CA170955-01A1 (M-Y Su, PI) 01/15/13–01/14/15
Volume and Morphology of Fibroglandular Tissue for Breast Cancer Risk
This project will evaluate the role of MRI-based density parameters, including the volume and the morphology of the fibroglandular tissue, and build a risk prediction model using a case-control study design.
Role: Co-investigator

5 R01 CA 195466-02 Tromberg (PI), Kelly (PI) 03/01/16-02/28/19
NIH/NCI
"Quantitative multiphoton microscopy for non-invasive diagnosis of melanoma"
This study will allow us to acquire sufficient clinical data to evaluate the ability of *in vivo* multiphoton microscopy (MPM) to provide quantitative optical imaging endpoints with high predictive power for non-invasive label-free diagnosis of pigmented lesions suspected of melanoma.
Role: Co-Investigator

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: James V. Jester

eRA COMMONS USER NAME (credential, e.g., agency login): JJESTER

POSITION TITLE: Professor of Ophthalmology and Biomedical Engineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Southern California, L.A., CA	B.S.	06/1972	Biology
University of Southern California, L.A., CA	Ph.D.	06/1978	Exp. Pathology
Estelle Doheny Eye Foundation, L.A., CA	PostDoc	07/1982	Ocular Pathology
National Eye Institute, Bethesda, MD	PostDoc	07/1983	Exp. Ocular Pathology

A. Personal Statement

I am an experimental pathologist with a major interest in understanding surface diseases. A major focus of my work has been on developing and evaluating novel imaging modalities for studying structure and cell function. I was involved in developing the first ophthalmic in vivo confocal microscope and I am now using non-linear optical microscopy to evaluate structure and function in situ and ex vivo. We have also recently developed a novel Immunofluorescent Computed Tomography (ICT) method for 3-dimensional reconstruction of tissue that combines NLO imaging with immunocytochemistry to quantitatively and volumetrically assess cell and protein distribution.

For this project, I will assist Dr. BenMohamed in performing and interpreting the histopathologic analysis of 6255 DRG and VMC tissues: We are requesting 0.36 calendar month time and effort for this confocal microscopy and imaging expert. Dr. Jester whose lab is adjacent to principal investigator lab will help confocal microscopy aspect of this proposal, including DRG and VMC tissues screening by microscopy. This includes (**1**) Confocal imaging of the sites of anti- HSV-2 pressure in DRG and VMC tissues; (**2**) Confocal microscopy three-dimensionally at high-resolution on a macroscopic scale of $CD4^+$ and $CD8^+$ T cell infiltrates into VMC herpetic lesions; and (**3**) Three-dimensionally at high-resolution on a macroscopic scale of $CD4^+$ and $CD8^+$ T cell infiltrates surrounding infected epithelial cells, fibroblasts/keratinocytes and neuronal axons in VMC and $CD4^+$ and $CD8^+$ T cell infiltrates surrounding neuronal body in the DRG and SSG SP of vaccinated and control 6255
Pertinent references are listed below.

1. Parfitt GJ, Xie Y, Reid KM, Dervillez X, Brown DJ, Jester JV: A novel immunofluorescent computed tomography (ICT) method to localise and quantify multiple antigens in large tissue volumes at high resolution. PLosOne 7:e53245, 2012.
2. Parfitt GJ, Xie Y, Geyfman M, Brown DJ, Jester JV: Absence of ductal hyper-keratinization in 6255 age-related meibomian gland dysfunction (ARMGD). Aging 11:825-834, 2013.
3. Parfitt GJ, Geyfman M, Xie Y, Jester JV: Characterization of quiescent epithelial cells in 6255 meibomian glands and hair follicle/sebaceous glands by immunofluorescence tomography. J Invest Dermatol 135: 1175-1177, 2015.

4. Parfitt GJ, Kavianpour B, Wu KL, Xie Y, Borwn DJ, Jester JV: Immunofluorescence tomography of 6255 ocular surface epithelial stem cells and the their niche microenvironment. Invest Ophthalmol Vis Sci 56: 7338-7344. 2015.

B. Positions and Honors

1974 - 1978	Hugh Edmundson Research Fellow, Dept. of Pathology USC Medical Center, Los Angeles
1981 - 1982	Instructor, Dept. of Ophthalmology & Pathology, USC/Los Angeles County Medical Center, Los Angeles
1982 - 1986	Asst. Prof., Dept. of Ophthalmology & Pathology, USC/Los Angeles County Medical Center, Los Angeles
1986 - 1991	Associate Professor of Ophthalmology & Pathology, Dept. of Ophthalmology/Center for Sight, Georgetown University Medical Center, Washington, DC
1991 - 2004	Professor of Ophthalmology, University of Texas, Southwestern Medical College, Dallas, Texas.
2004 – present	Professor of Ophthalmology and Biomedical Engineering, University of California, Irvine, Irvine, California.
2007 – present	Jack H. Skirball Endowed Chair

Membership on Federal Government Advisory Committees

1989-1991	Ad hoc grant reviewer for NIH VIS A Study Section
1989-1991	Member of Special Study Section -2 for Small Business Innovative Research (SBIR)
1997-1998	Member of the Peer Review Panel on Photorefractive Keratectomy Research for the US Army Medical Research and Materiel Command.
2002-2004	Ad hoc grant reviewer for NIH VIS A Study Section
2004-present	Member of NIH/NEI Anterior Eye Diseases Study Panel
2009	Member of ICCVAM/NICEATM Regulatory Advisory Panel.
2012	Member of the NIH, Neuroscience and Ophthalmic Imaging Technologies Study Section.
2012	Member, Department of Defense, Vision Research Panel Review, American Institute of Biological Sciences.

Awards

1981	Fight for Sight Research Award.
1986	Research Manpower Award, Research to Prevent Blindness, Inc., New York, NY.
1994	Senior Scientist Award, Research to Prevent Blindness, Inc., New York, NY.
2003	2nd Senior Scientist Award, Research to Prevent Blindness, Inc., New York, NY.
2009	ARVO Gold Fellow
2010	Founders Award, Wavefront & Presbyopic Refractive Corrections.
2013	Career Achievement Award, Ocular Toxicology Specialty Section, San Antonio, Texas, March 13, 2013
2017	Thygeson Lecture, Ocular Microbiology and Immunology Group, New Orleans, November 10, 2017.

C. Contribution to Science

1. My laboratory was to first to develop a [redacted] of meibomian gland dysfunction (MGD) and establish a link between hyperkeratinization and ductal plugging of the meibomian gland leading to an experimental model of MGD. As part of this work, we published a novel infrared photography approach to documenting changes in the meibomian gland that today has been modified by others to assess MGD in patients with Dry Eye. Through collaboration with Dr. William Mathers, we later showed that loss of meibomian glands led to increased tear osmolarity and the development of signs and symptoms of Dry Eye. Today, it is widely recognized the MGD is a major cause of Dry Eye disease and is the most common complaint of patients visiting optometric and ophthalmic practices. More recently we have established a 6255 model

of age-related MGD that develops dropout of meibomian glands similar to that observed in older Dry Eye patients that does not involve gland hyper-keratinization (1a,1b), suggesting that meibomian gland cell renewal may play a role in the development evaporative dry eye associated with MG dropout. Towards investigating this theory, we have recently identified and quantified label-retaining cells in the 6255 meibomian glands and shown that environmental stress leads to up-regulation of cell proliferation (1c). Importantly, hyperproliferation of the meibomian gland may also be associated with direct changes in the quality of the meibomian gland lipid and not hyperkeratinization as reviewed in a recent paper (1d).

1a) Nien CH, Massei S, Lin G, Nabavi C, Tao J, Brown DJ, Paugh JR, Jester JV: Effects of age and dysfunction on human meibomian glands. Arch Ophthalmol 129, 462-469, 2011. PMCID: in progress
1b) Parfitt GJ, Xie Y, Geyfman M, Brown DJ, Jester JV: Absence of ductal hyper-keratinization in 6255 age-related meibomian gland dysfunction (ARMGD). Aging 11:825-834, 2013. PMCID: PMC3868725
1c) Parfitt GJ, Lewis P, Young RD, Richardson A, Lyons JG, Di Girolamo N, Jester JV: Renewal of holocrine meibomian glands by label-retaining, uni-potent epithelial progenitors. Stem Cell Reports 7:399-410, 2016.
1d) Hwang HS, Parfitt GJ, Brown DJ, Jester JV: Meibocyte differentiation and renewal: Insights into novel mechanisms of meibomian gland dysfunction (MGD). Exp Eye Res 163:37-45, 2017.

2. Early my career I was recruited to Georgetown University to help in the development of an in vivo confocal microscope for evaluating corneal cell biology in live 6255 and human subjects. A development team that I helped assemble included Dr. Dwight Cavanagh as a clinician scientist, Dr. Matt Petroll, a Biomedical Engineer with expertise in digital image processing, and myself. Some of the first high-resolution images of living cells using the microscope we developed were published in 1991 (2a), which showed the potential of this new microscopic paradigm for use in clinical diagnosis and treatment of corneal disease (2b). Later we developed novel quantitative approaches to measuring corneal sub-layer thickness using the in vivo confocal microscope (2c), which proved valuable in assessing the response of the cornea to new refractive surgical procedures, particularly photorefractive keratectomy (2d). Today, in vivo confocal microscopy is widely recognized as an important

2a) Jester JV, Petroll WM, Andrews P, Cavanagh HD, Lemp MA: In vivo confocal microscopy. J Elect Micros Tech 18:50-60, 1991.
2b) Cavanagh HD, Petroll WM, Alizadeh H, He Y-G, McCulley JP, Jester JV: Clinical and diagnostic use of in vivo confocal microscopy in patients with corneal disease. Ophthalmol 100: 1444-1454, 1993.
2c) Li H-F, Petroll WM, Maurer JK, Cavanagh HD, Jester JV: Epithelial and corneal thickness measurements by in vivo confocal microscope through focusing (CMTF). Curr Eye Res 16:214-221, 1997.
2d) Moller-Pedersen T, Cavanagh HD, Petroll WM, Jester JV: Stromal wound healing explains refractive instability and haze development after photorefractive keratectomy. A one-year confocal microscopic study. Ophthalmology 107:1235-1245, 2000.

3. Prior to my studies of corneal wound healing, it was generally thought that corneal wounds did not undergo wound contraction similar to skin. My laboratory was the first to establish that corneal wound fibroblasts developed contractile and ultrastructural features consistent with skin myofibroblasts (3a). My laboratory was also to first to establish a serum-free in vitro culture system that maintained the corneal keratocyte phenotype, and showed that the wound cytokine, TGFβ, induced expression of α-smooth muscle actin, the biomarker for myofibroblast differentiation (3b). Later studies in a rabbit wound healing model showed that topical treatment of corneal wounds with neutralizing antibodies to TGFβ blocked corneal wound fibrosis and maintained corneal clarity. These studies lead to the discovery of keratocyte crystallin proteins, ALDH1A1 and ALDH3A1, that are abundantly expressed in normal corneal keratocytes, but are marked down-regulated in wound healing fibroblasts and myofibroblasts. This finding has lead to the now popular hypothesis that keratocyte crystallin proteins regulate corneal transparency at the cellular level (3d).

3a) Jester J.V., Rodrigues, M.M., Herman, I.M.: Characterization of avascular corneal wound healing fibroblast. New insights into the myofibroblast. Am. J. Pathol. 127:140-148, 1987.
3b) Jester JV, Barry PA, Cavanagh HD, Petroll WM: Induction of ✔-smooth muscle actin (✔-SM) expression and myofibroblast transformation in cultured keratocytes. Cornea 15:505-516, 1996.

3c) Moller-Pedersen T, Petroll WM, Cavanagh HD, Jester JV: Neutralizing antibody to TGF modulates stromal fibrosis but not regression of photoablative effect following PRK. Curr Eye Res 17:736-747, 1998.

3d) Jester JV: Corneal crystallins and the development of cellular transparency. Sem Cell & Devel Biol 19:82-93, 2008.

4. My more recent work has focused on using non-linear optical imaging of second harmonic generated signals (NLO-SHG) to evaluate corneal collagen organization. Using this non-invasive imaging paradigm we were the first to show that NLO-SHG can be used to detect differences in the lamellar organization of collagen in Keratoconus patients compared to normal corneas, which involved the loss of lamellae that inserted into the anterior limiting lamina Bowman's layer (4a). To explore in more depth the significance to these differences, we developed a high-resolution macroscopic approach to imaging the corneal stroma using NLO-SHG that allow for the tracking of single collagen lamellae throughout the length and depth of the cornea (4b). In these studies we showed that the normal human cornea contained 'transverse' collagen lamellae that intertwined with other collagen lamellae and inserted into Bowman's layer. Importantly, these transverse lamellae and lamellar intertwining is highest in the anterior stroma, which we and others have now shown is biomechanically the stiffest region of the cornea. Furthermore, lamellar intertwining and branching seem to be a defining characteristic of corneal development during evolution, with higher vertebrate corneas showing increasing branching combined with increasing mechanical stiffness to control corneal shape (4c). These findings suggest that changes in the macroscopic organization of collagen lead to mechanical weaken and ectasia as observed in Keratoconus. These findings also suggest that mechanical stiffness of the collagen fibers may regulate corneal shape, and that controlling regional corneal stiffness may provide a novel therapeutic strategy for treating refractive errors of the cornea without removal of corneal tissue. To explore test this hypothesis, we are currently developing an NLO corneal crosslinking approach to regionally change corneal stiffness to treated both Keratoconus and potentially other refractive errors (4d).

4a) Morishige N, Wahlert AJ, Kenney MC, Brown DJ, Kawamoto K, Chikama T-I, Nishida T, Jester JV: Second Harmonic Imaging Microscopy of Normal Human and Keratoconus Cornea. Invest Ophthalmol Vis Sci 48: 1087-1094, 2007.

4b) Jester JV, Winkler M, Jester BE, Nien C, Chai D, Brown DJ: Evaluating corneal collagen organization using high resolution non linear optical (NLO) macroscopy. Eye & Contact Lens 36:260-264, 2010.

4c) Koudouna E, Winkler M, Mikula E, Juhasz T, Brown DJ, Jester JV: Evolution of the vertebrate corneal stroma. Prog Ret Eye Res 2018 Feb1, doi: 10..1016/jpreteyeres.2018.01.002.

4d) Bradford SM, Mikula ER, Chai D, Brown DJ, Juhasz T, Jester JV: Custom built nonlinear optical crosslinking (NLO CXL) device capable of producing mechanical stiffening in ex vivo rabbit corneas. Biomed Opt Express 8:4788-4797, 2017.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/james.jester.1/bibliograpahy/41149116/public/?sort=date&direction=descending

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D. Research Support

Ongoing Research Support

R01 EY021510 Jester (PI) 09/30/2011 to 08/31/2020
Age-Related Meibomian Gland Dysfunction
The specific aims of this project is to evaluate the effects of age on meibomian gland functions and signal transduction by the lipid sensitive nuclear receptor, peroxisome proliferator-activated receptor-γ (PPARγ)
Role: PI

R21 AR069963-02 Anderson (PI) 05-17-17 to 03-31-19
An epidermal mechanism for digit separation.
Role: Co-PI

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Read, Elizabeth L.

eRA COMMONS USER NAME (credential, e.g., agency login): elizabethread

POSITION TITLE: Assistant Professor of Chemical and Biomolecular Engineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Colorado, Boulder	B.A.	05/2003	Chemistry
University of Colorado, Boulder	B.A.	05/2003	Mathematics
University of California, Berkeley	Ph.D.	05/2008	Chemistry
Massachusetts Institute of Technology	Postdoc	06/2012	Chemical Engineering

A. Personal Statement

My research is in mathematical modeling and computer simulation of cellular behavior at multiple scales. My group specializes in three areas: (1) Mathematical modeling of stochastic processes in cell biology, (2) Multi-scale stochastic simulation methods, (3) Data-model integration using statistical inference and model selection. We apply these techniques to a number of cell-biological questions in collaboration with experimentalists. Current areas of study include DNA methylation dynamics, gene regulatory network dynamics, and immune cell dynamics (including T cells and macrophages). My home department is in the Chemical and Biomolecular Engineering Department. I am active also in the Center for Complex Biological Systems, the NSF Simons Center for Multiscale Cell Fate, and am affiliated with the Molecular Biology and Biochemistry Department at UCI. My group specializes in mathematical modeling and simulation methods of cellular processes using a variety of mathematical frameworks and software platforms. We will leverage these tools for this project. Furthermore, in preliminary work we have developed software codes implementing models of HSV virus dynamics and multi-sub-population T cell responses.

For this project, I will assist Dr. BenMohamed by adapting mathematical models to study functional/dysfunctional HSV-specific $CD4^+$ and $CD8^+$ T cell responses in the 6255 that receive various combinations of the prime/pull vaccine. I will test and refine these mathematical models, in an iterative process, on the basis of experimental results provide by Dr. BenMohamed's lab.

This will include: (**1**) Developing mathematical models to capture the $CD4^+$ and $CD8^+$ T_{RM} cell dynamics of the containment within HSV-2 infected DRG and VMC. This includes mathematical models of HSV-2 reactivation from DRG based on observed patterns of single neuron loads and $CD4^+$ and $CD8^+$ T cell infiltration and HSV-2 shedding rate from VMC; and (**2**) Develop mathematical models to characterize the duration of protection, and the protective mechanisms induced by the prime/pull vaccine in the 6255 model. In doing so, we expect the mathematical modeling, together with the *in vitro*, *in situ, ex vivo*, and *in vivo* results, to shed more light the relative contribution of the peripheral epithelial T cell immunity epithelium vs. central neuronal T cell immunity in protection against recurrent genital herpes. This will help optimize the therapeutic prime/pull vaccine strategies.

B. Positions and Honors

Positions and Employment

2008-2009	Postdoctoral Researcher, Dept. of Chemical Engineering, Mass. Institute of Technology
2009-2012	Postdoctoral Fellow, Dept. of Chemical Engineering, Mass. Institute of Technology
2012-present	Assistant Professor, Dept. of Chemical Engineering and Materials Science, University of California, Irvine
2014-present	Assistant Professor, Dept. of Molecular Biology and Biochemistry, UC Irvine (by courtesy)

Other Experience and Professional Memberships

2010-2012	Faculty of 1000 Associate Faculty Member
2013-present	Member, Biophysical Society
2017-present	Member, American Institute of Chemical Engineers

Honors

2009-2012	Frederic M. Richards Postdoctoral Fellow of the Jane Coffin Childs Memorial Fund
2015	Professor of the Year Award, ChEMS, UCI Engineering Student Council
2015	Dean's Honoree for Excellence in Undergraduate Education, HSSoE
2018	Scialog Foundation Fellow 2018: Chemical Machinery of the Cell

C. Contribution to Science

1. Rare-Event Simulation Approaches to Study Biological Network Dynamics

My group has developed novel computational methods for simulation and analysis of biological network dynamics. These "rare-event" methods increase the computational efficiency of simulations, when challenged by processes that span multiple timescales. Our approach adapts theoretical ideas and computational algorithms from the fields of Chemical Physics and Molecular Dynamics, in order to tackle computational challenges at the systems scale. Effectively, these methods extend the capability of stochastic simulations in cell biology. We have used these methods to investigate multi-stability and stochastic phenotype transitions in gene networks, with potential applications in stem cell reprogramming and cancer therapy, as well as hydrodynamics of cell-cell contact.

a. M. J. Tse, B. K. Chu, C. P. Gallivan**, E. L. Read**. Rare-event sampling of epigenetic landscapes and phenotype transitions. *PLOS Computational Biology* 14(8): e1006336 (2018).
b. B. K. Chu, M. J. Tse, R. R. Sato, **E. L. Read**. Markov State Models of Gene Regulatory Networks. *BMC Systems Biology* 11(1):14 (2017).
c. M. J. Tse, B. K. Chu, M. Roy, **E. L. Read**. DNA-Binding Kinetics Determines Mechanism of Noise-Induced Switching in Gene Networks. *Biophys. J*. 109 (8) 1746-57 (2015).
d. K. Liu, B. K. Chu, J. Newby, **E. L. Read**, J. Lowengrub, J. Allard, Hydrodynamics of transient cell-cell contact: The role of membrane permeability and active protrusion length. *PLOS Computational Biology* (*Accepted*, 2019).

2. Mathematical Modeling of Immune Cell Dynamics

One area of my research is in mathematical modeling of immune cell dynamics, both at the intra-cellular and population level. My postdoctoral research advanced understanding of the dynamics of T cell immune responses in HIV infection. Integrating approaches from mathematical biology and stochastic chemical kinetics in collaboration with leading HIV immunologists at the Ragon Institute, this work led to a novel mechanistic hypothesis regarding how thymic selection during T cell repertoire development influences HIV viral dynamics in infection. In related work, we showed how the rare occurrence of natural immune control over HIV could be related to stochastic dynamics of HIV evolution under immune pressure. In my group at UCI, we developed a mathematical model of macrophage polarization in collaboration with Prof. Wendy Liu, and proposed a core regulatory logic that can recapitulate the dynamic macrophage response to mixed activation signals.

a. **E. L. Read**, A. A. Tovo-Dwyer, A. K. Chakraborty. Stochastic effects are important in intra-host HIV evolution even when viral loads are high. *Proc. Natl. Acad. Sci.* 109, 19727-19732 (2012).
b. A. Košmrlj*, **E. L. Read*,** Y. Qi, T. M. Allen, M. Altfeld, S. G. Deeks, F. Pereyra, M. Carrington, B. D. Walker, A. K. Chakraborty. Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection. *Nature* 465, 350-354 (2010).
c. Smith, T. D., Tse, M. J., **Read, E. L.**, Liu, W. Regulation of macrophage polarization and plasticity by complex activation signals. *Integrative biology: 8(9)*, 946-55 (2016).
d. Y. Vodovotz, A. Xia, **E. L. Read**, J. Bassaganya-Riera, D. A. Hafler, E. Sontag, J. Wang, J. S. Tsang, J. D. Day, S. Kleinstein, A. J. Butte, M. C Altman, R. Hammond, C. Benoist, and S. C. Sealfon. Solving Immunology? *Trends in Immunology* 38(2) 116:127 (2017).

3. Theory and Experiments to Elucidate Dynamics of Photosynthetic Light Harvesting
My Ph.D. work contributed new insights into how the architecture of photosynthetic pigment-protein complexes governs the efficiency of light capture. For this work, we combined state of the art spectroscopic techniques (2D electronic spectroscopy) with theoretical modeling. I developed codes to model spectroscopic data under multiple experimental protocols, along with data-fitting tools, in order to infer how energetic coupling in pigment protein complexes determines the dynamics of energy transfer, which is critical to the efficiency of photosynthetic light-harvesting in plants and bacteria. This work led to the discovery of quantum coherence in biological systems, and elucidated the link between growth conditions of photosynthetic bacteria, the structures of their pigment-protein antennae, and efficiency of light-harvesting.

a. **E. L. Read**, G. S. Schlau-Cohen, G. S. Engel, T. Georgiou, M. Z. Papiz, G. R. Fleming. Pigment organization and energy level structure in Light-harvesting Complex 4: Insights from two-dimensional electronic spectroscopy. *J. Phys. Chem. B* 113, 6495-6504 (2009).
b. **E. L. Read**, G. S. Schlau-Cohen, G. S. Engel, R. E. Blankenship, G. R. Fleming. Visualization of excitonic structure in the Fenna-Matthews-Olson photosynthetic complex by polarization-dependent two-dimensional electronic spectroscopy. *Biophys. J.* 95, 847-856 (2008).
c. **E. L. Read***, G. S. Engel*, T. R. Calhoun, T. Mančal, T. K. Ahn, R. E. Blankenship, and G. R. Fleming. Cross-peak-specific two-dimensional electronic spectroscopy. *Proc. Natl. Acad. Sci.* 104, 14203-14208 (2007).
d. G. S. Engel, T. R. Calhoun, **E. L. Read**, T. K. Ahn, T. Mančal, Y.-C. Cheng, R. E. Blankenship, G. R. Fleming. Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems. *Nature* 446, 782-786 (2007).

*equal contributors

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/16E5pH8RhgqQA/bibliograpahy/48024592/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

NSF DMS-1715455 Mathematical Biology 09/01/17-08/31/20
"Collaborative Research: Spatial stochastic rare events by asymptotics and weighted ensemble sampling to understand how cells make space"
The goal of this project is to develop computational algorithms and software that will enable efficient simulation of spatial, stochastic systems relevant to cell biology.
Role: PI

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ahmed, Rafi

eRA COMMONS USER NAME (credential, e.g., agency login): RAHMED

POSITION TITLE: Director, Emory Vaccine Center

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Osmania University, Hyderabad, India	B. Sc.	1968	Chemistry
Idaho State University, Pocatello, Idaho	B.S./M.S.	1972/1974	Microbiology
Harvard University, Cambridge, MA	Ph.D.	1981	Micro. & Molec. Gen.
Scripps Clinic & Research Foundation	Post-doc	1981-84	Immunology

A. Personal Statement

My research efforts are directed towards: **1**. Understanding the mechanisms of immunological memory and using this knowledge to develop new and more effective vaccines. **2**. Defining the mechanisms of T cell exhaustion during chronic viral infections and cancer and developing strategies for restoring function in exhausted T cells.

I am collaborating with Dr. BenMohamed on projects of herpes T cells and help analyze HSV-specific $CD8^+$ $T_{CM}/T_{EM}/T_{RM}$ cell subsets associated with symptomatic genital herpes.

B. Positions and Honors

Positions and Employment

1984 Assistant Member, Dept. Immunology, Scripps Clinic and Research Foundation, La Jolla, CA
1984-1988 Assistant Professor, Dept. Microbiology and Immunology, UCLA School of Medicine
1988-1992 Associate Professor, Dept. Microbiology and Immunology, UCLA School of Medicine
1992-1995 Professor, Dept. of Microbiology and Immunology, UCLA School of Medicine
1995-Present Georgia Research Alliance Professor of Vaccinology, Emory University
1995-Present Director, Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA

Honors

2004 Fellow, American Academy of Microbiology
2009 Member, National Academy of Sciences (USA)
2013 Foreign Member, Indian National Academy of Sciences
2014 Member, National Academy of Medicine (USA)
2015 AAI Excellence in Mentoring Award

Scientific Advisory Boards

1998-Present SAB, Ministry of Science, Dept. of Biotechnology, Gov. of India, New Delhi, India
2006-Present Scientific Advisory Board, Singapore-MIT Alliance for Research & Technology, Cambridge, MA
2007-Present NIH Vaccine Research Center, Board of Scientific Counselors, Bethesda, MD
2008-Present Immune Design, Seattle WA

2008-Present Selecta Biosciences, Boston, MA
2014–Present Scientific Advisory Board, La Jolla Institute for Allergy & Immunology, La Jolla, CA
2014-Present Merck Pharmaceutical, West Point, PA

C. Contribution to Science

Dr. Ahmed's work has been highly influential in shaping our current understanding of memory T cell differentiation and anti-viral T and B cell immunity. Dr. Ahmed's group revealed mechanisms by which long-term immune memory is maintained after vaccination and defined the underlying causes of T cell dysfunction during chronic infection. Dr. Ahmed's work identified PD-1 as a major regulator of T cell exhaustion. This work has resulted in the development of strategies for the treatment of human chronic infections and cancer.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/rafi.ahmed.1/bibliography/41160415/public/?sort=date&direction=ascending.

D. Research Support (Active)

1U01AI115651 (Ahmed) 12/01/14 – 11/30/19
ICIDR: Dengue Virus Infection in India
The major goal of this grant is to: (i) build capacity for dengue research in India using state of the art tools and technologies; and (ii) To address critical scientific questions important to the health and well being of dengue exposed population in India.

5 U19 AI057266 (Ahmed) 09/01/03-04/30/19
NIH/NIAID
Vaccine Induced Immunity in the Young and Aged
This is a Human Immunology Center grant to support studies characterizing immunological memory in humans after vaccination.

1 U19 AI090023 (Pulendran) 07/12/2010-06/30/20
NIH/NIAID
System Biological Analyses of Innate and Adaptive Responses to Vaccination
The major goal of this project to use a "systems vaccinology" approach to probe the immune response to vaccination in special populations, who are at the "extremes of age," and immunocompromised. This project will evaluate the adaptive immune responses to the VZV and pneumococcal vaccines.

HHSN266200700006C (Orenstein) 03/2007-03/29/21
NIH/NIAID
NIAID Center of Excellence for Influenza Research and Surveillance
The major goal of this contract is to characterize the humoral response to the influenza vaccine.

5 P01 AI056299 (Sharpe) 09/30/03-08/31/19
Harvard University (NIH/NIAID)
T Cell Costimulatory Pathways: Functions and Interactions
The major goal of this project is to analyze how PD-1 and its ligands regulate humoral immunity during acute viral infection.

Completed Research Support

1 P01 AI080192 Ahmed (PI) 09/22/08-08/31/13
NIH/NIAID
PD-1 Function, Signaling, and Regulation During Viral Infection.
The major goal of this grant was to define the role of the PD-1 pathway in regulating viral control during chronic viral infection.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		LBACHIR		BENMOHAMED		PD/PI	189,600.00	6			94,800.00	22,266.00	117,066.00
2 .		CHRISTINE		MCLAREN		Co-Investigator	185,292.00	0.6			9,265.00	4,572.00	13,837.00
3 .		ELIZABETH		READ		Co-Investigator	155,411.00	0.3			3,885.00	1,037.00	4,922.00
4 .		JAMES		JESTER		Co-Investigator	189,600.00	0.36			5,688.00	1,724.00	7,412.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **143,237.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	18			85,872.00	35,650.00	121,522.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Data Analyst	1.2			8,426.00	4,686.00	13,112.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**134,634.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**277,871.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,500.00
2. Foreign Travel Costs	
Total Travel Cost	**6,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	36,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	81,900.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Purchase & Husbandry	69,137.00
Total Other Direct Costs	**189,537.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**473,908.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	54.5	417,008.00	227,269.00
		Total Indirect Costs	**227,269.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Robert W. Lee, (415) 437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**701,177.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	701,177.00

L. Budget Justification* File Name: BudgetJustificationGP_04_11_20191011294176.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		LBACHIR		BENMOHAMED		PD/PI	189,600.00	6			94,800.00	22,266.00	117,066.00
2 .		CHRISTINE		MCLAREN		Co-Investigator	189,600.00	0.6			9,480.00	4,678.00	14,158.00
3 .		ELIZABETH		READ		Co-Investigator	160,073.00	0.3			4,002.00	1,069.00	5,071.00
4 .		JAMES		JESTER		Co-Investigator	189,600.00	0.36			5,688.00	1,724.00	7,412.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **143,707.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	18			88,448.00	37,137.00	125,585.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Data Analyst	1.2			8,679.00	4,827.00	13,506.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**139,091.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**282,798.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT12838353 Funding Opportunity Number: PA-19-056 . Received Date: 2019-04-11T18:17:49.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,605.00
2. Foreign Travel Costs	
Total Travel Cost	**6,605.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	37,595.00
2. Publication Costs	2,060.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	81,900.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Purchase & Husbandry	72,594.00
Total Other Direct Costs	**194,149.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**483,552.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	54.5	401,652.00	218,900.00
		Total Indirect Costs	**218,900.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Robert W. Lee, (415) 437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**702,452.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	702,452.00

L. Budget Justification* File Name: BudgetJustificationGP_04_11_20191011294176.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		LBACHIR		BENMOHAMED		PD/PI	189,600.00	6			94,800.00	22,266.00	117,066.00
2 .		CHRISTINE		MCLAREN		Co-Investigator	189,600.00	0.6			9,480.00	4,678.00	14,158.00
3 .		ELIZABETH		READ		Co-Investigator	164,875.00	0.3			4,122.00	1,101.00	5,223.00
4 .		JAMES		JESTER		Co-Investigator	189,600.00	0.36			5,688.00	1,724.00	7,412.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **143,859.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	18			91,102.00	38,732.00	129,834.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Data Analyst	1.2			8,939.00	4,971.00	13,910.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**143,744.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**287,603.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,713.00
2. Foreign Travel Costs	
Total Travel Cost	**6,713.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	38,723.00
2. Publication Costs	2,122.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Purchase & Husbandry	76,224.00
Total Other Direct Costs	**117,069.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**411,385.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	54.5	411,385.00	224,205.00
		Total Indirect Costs	**224,205.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Robert W. Lee, (415) 437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**635,590.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	635,590.00

L. Budget Justification* File Name: BudgetJustificationGP_04_11_20191011294176.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		LBACHIR		BENMOHAMED		PD/PI	189,600.00	6			94,800.00	22,266.00	117,066.00
2 .		CHRISTINE		MCLAREN		Co-Investigator	189,600.00	0.6			9,480.00	4,678.00	14,158.00
3 .		ELIZABETH		READ		Co-Investigator	169,821.00	0.3			4,246.00	1,134.00	5,380.00
4 .		JAMES		JESTER		Co-Investigator	189,600.00	0.36			5,688.00	1,724.00	7,412.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **144,016.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	18			93,835.00	40,380.00	134,215.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Data Analyst	1.2			9,207.00	5,120.00	14,327.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**148,542.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**292,558.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-04-11T18:17:49.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,825.00
2. Foreign Travel Costs	
Total Travel Cost	**6,825.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	39,885.00
2. Publication Costs	2,185.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Purchase & Husbandry	80,035.00
Total Other Direct Costs	**122,105.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**421,488.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	54.5	421,488.00	229,711.00
		Total Indirect Costs	**229,711.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Robert W. Lee, (415) 437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**651,199.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	651,199.00

L. Budget Justification* File Name: BudgetJustificationGP_04_11_20191011294176.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		LBACHIR		BENMOHAMED		PD/PI	189,600.00	6			94,800.00	22,266.00	117,066.00
2 .		CHRISTINE		MCLAREN		Co-Investigator	189,600.00	0.6			9,480.00	4,678.00	14,158.00
3 .		ELIZABETH		READ		Co-Investigator	174,916.00	0.3			4,373.00	1,168.00	5,541.00
4 .		JAMES		JESTER		Co-Investigator	189,600.00	0.36			5,688.00	1,724.00	7,412.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **144,177.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	18			96,650.00	41,963.00	138,613.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Data Analyst	1.2			9,484.00	5,274.00	14,758.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**153,371.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**297,548.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT12838353 Funding Opportunity Number: PA-19-056 . Received Date: 2019-04-11T18:17:49.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,939.00
2. Foreign Travel Costs	
Total Travel Cost	**6,939.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 046705849

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Irvine

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	41,081.00
2. Publication Costs	2,251.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Purchase & Husbandry	84,037.00
Total Other Direct Costs	**127,369.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**431,856.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	54.5	431,856.00	235,362.00
		Total Indirect Costs	**235,362.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Robert W. Lee, (415) 437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**667,218.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	667,218.00

L. Budget Justification* File Name: BudgetJustificationGP_04_11_20191011294176.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

KEY PERSONNEL

Lbachir BenMohamed, Ph. D. **Principal Investigator** **6.0 Calendar Months**

Dr. BenMohamed (Professor of immunology and Director of Cellular and Molecular Immunology Laboratory) is requesting 6.0 calendar months' time and effort to supervise members of Cellular and Molecular Immunology Laboratory personnel working on this project, as well as actively participate in designing producing the prime/pull vaccine constructs, antibodies and peptide inhibitors to block the immune checkpoints in the 6255 6255 model of recurrent genital herpes. He is a full faculty member at Gavin Herbert Eye Institute and the Institute for Immunology, University of California Irvine (UC Irvine). He is an expert in herpes infection, immunity and inflammation in both 6255 models and in humans. He has over 25-year background in cellular and molecular immunology, and is a leading researcher in genital and ocular herpes sub-unit vaccine development. For the last 25 years, the principal investigator has accumulated extensive research experience in the field of immunity, inflammation and immune- evasion from The Pasteur Institute Paris, The City of Hope National Medical Center (Duarte, CA), Cedars Sinai Medical Center (Los Angeles, CA) and for last 15 years as the founder and head of the Laboratory of Cellular and Molecular Immunology, at UC Irvine.

The Principal investigator primary role in the proposed study will be to assume overall responsibility for designing, coordinating and conducting the vaccine research described in this project. The PI, together with the co-PIs and collaborators Christine McLaren, Dr. Elizabeth Read, Dr. James Jester consultant Dr. Rafi Ahmed, will be involved in the analysis of the immune mechanisms, immunotherapy & immune checkpoint blockade to prevent genital herpes, as described in the proposal. The principal investigator will also be responsible for preparing manuscripts based on data resulting from the proposed study. The principal investigator will be assisted by two post-doctoral fellow, Dr. Ruchi Srivastava and Dr. Swayam Prakash, that worked with Dr. BenMohamed for past 5 years, as well as Dr. Angele Nalbandian, a data analyst, to perform the *in silico, in vitro*, *in situ, ex vivo*, and [redacted] assays described in this proposal.

Christine McLaren, Ph. D. **Co-Investigator** **0.60 Calendar Months**

We are requesting 0.6 calendar month time and effort for Dr. McLaren, the Director of Biostatistics at the Department of Epidemiology (UC, Irvine). Dr. McLaren is a professor of Epidemiology and Bio-statistics at UC Irvine and will help with the statistical analysis as described in this application. Dr. BenMohamed and Dr. McLaren have been collaborating for the last 6 years on many ongoing herpes immunology projects. Dr. McLaren will help analyze the statistics of the *in vitro*, *in situ, ex vivo*, and [redacted] results. She will also analyze the statistics comparing the contribution of the peripheral epithelial T cell immunity (at the VMC) epithelium vs. central neuronal T cell immunity (at the DRG) in protection against recurrent genital herpes. This will include: (**1**) Statistical analysis to capture the $CD4^+$ and $CD8^+$ T_{RM} cell dynamics of the containment within HSV-2 infected DRG and VMC. This includes statistical analysis of HSV-2 reactivation from DRG based on observed patterns of single neuron loads and $CD4^+$ and $CD8^+$ T cell infiltration and HSV-2 shedding rate from VMC; and (**2**) Statistical analysis to characterize the duration of protection, and the protective mechanisms induced by the prime/pull vaccine in the 6255 model. In doing so, we expect the statistical analysis, to help determine relative contribution of the peripheral epithelial T cell immunity epithelium vs. central neuronal T cell immunity in protection against recurrent genital herpes.

Elizabeth Read, Ph.D. **Co-Investigator** **0.30 Calendar Months**

We are requesting 0.30 summer month time and effort for Dr. Elisabeth Read, an associate professor of Mathematical Modeling at UC Irvine and a member of the Center for Complex Biological Systems. Her group has developed mathematical theories and software tools for data-driven modeling of cellular immune responses and inflammation. For this project, she will assist Dr. BenMohamed by adapting these tools to the study of functional/dysfunctional HSV-specific $CD4^+$ and $CD8^+$ T cell responses in the 6255 that receive various combinations of the prime/pull vaccine. She will test and refine these mathematical models, in an iterative process, on the basis of experimental results provide by Dr. BenMohamed's lab. This will include: (**1**) Developing mathematical models to capture the $CD4^+$ and $CD8^+$ T_{RM} cell dynamics of the containment within HSV-2 infected DRG and VMC. This includes mathematical models of HSV-2 reactivation from DRG based on

observed patterns of single neuron loads and CD4+ and CD8+ T cell infiltration and HSV-2 shedding rate from VMC; and (**2**) Develop mathematical models to characterize the duration of protection, and the protective mechanisms induced by the prime/pull vaccine in the 6255 model. In doing so, we expect the mathematical modeling, together with the *in vitro*, *in situ, ex vivo*, and *in vivo* results, to shed more light the relative contribution of the peripheral epithelial T cell immunity epithelium vs. central neuronal T cell immunity in protection against recurrent genital herpes. This will help optimize the therapeutic prime/pull vaccine strategies.

James V. Jester, Ph. D. **Co-Investigator** **0.36 Calendar Months**

We are requesting 0.36 calendar month time and effort for this confocal microscopy and imaging expert. Dr. Jester whose lab is adjacent to principal investigator lab will help confocal microscopy aspect of this proposal, including DRG and VMC tissues screening by microscopy. He is an imaging specialist at UC Irvine. Dr. Jester will devote effort as needed. He will be available on an as needed basis to help with performing and analyzing the confocal microscopy experiments. This includes (**1**) Confocal imaging of the sites of anti- HSV-2 pressure in DRG and VMC tissues; (**2**) Confocal microscopy three-dimensionally at high-resolution on a macroscopic scale of CD4+ and CD8+ T cell infiltrates into VMC herpetic lesions; and (**3**) Three-dimensionally at high-resolution on a macroscopic scale of CD4+ and CD8+ T cell infiltrates surrounding infected epithelial cells, fibroblasts/keratinocytes and neuronal axons in VMC and CD4+ and CD8+ T cell infiltrates surrounding neuronal body in the DRG of vaccinated and control 6255 as illustrated in **Fig. 9**.

Rafi Ahmed, Ph.D., a professor of immunology and the director at the Emory Vaccine Center will be actively involved in this project. Dr. Ahmed's will travel to UCI each year for consulting, presentation, and analysis of the project.

NON-KEY PERSONNEL

Ruchi Srivastava Ph. D. **Post-doctoral fellow** **12 Calendar Months**

We are requesting 12 calendar month time and effort for the Post-doctoral fellow, Dr. Srivastava has been working on in cellular and molecular immunology and inflammation projects in Dr. BenMohamed laboratory for past 5 year. Her principal task will be to carry out in vitro and in vivo immunological experiments described in this application. Specifically, she will be responsible for synthetic peptide handling and storage, cell culture, flow cytometry assay, Luminex assay, cell sorting, generation of antigen presenting cells, T-cell functional assays, RNASeq assay, pro- and anti-inflammatory cytokine Luminex and ELISPOT assays, FACS and confocal microscopy as described in this project.

Swayam Prakash Ph. D. **Post-doctoral fellow** **6 Calendar Months**

We are requesting 6 calendar month time and effort for an additional Post-doctoral fellow. As part of his dissertation research under Dr. BenMohamed' mentorship he performed single-cell RNA-Seq data studies

6255

6255

Angele Nalbandian Ph. D. **Data Analyst** **1.2 Calendar Months**

We are requesting 1.2 calendar month time for Angele Nalbandian to provide data analysis for the project. She will be involved in analyzing the single cell scRNASeq data of CD4+ and CD8+ T cells from 6255 that were immunized with each of the 19 vaccine candidates as well as controls.

Melanie Oakes, Ph. D. **Collaborator**

For the single-cell transcriptional profiling aspect of this proposal, there is Dr. Oakes. She is a RNASeq specialist at UCI and has over 20 years in transcriptional profiling. She will be available on an as needed basis to help with integration and analysis the single-cell sequencing data. Dr. Oakes, together with Dr. Wu, co-led the single-cell transcriptional profiling core at UCI

Jenny Wu, Ph. D. **Collaborator**

Dr. Wu, a Bioinformaticist, will lead the computational analyses of single-cell RNA-Seq data, and will design and oversee the interpretation of the proposed single-cell transcriptional profiling research together with Dr. Oakes and Dr. BenMohamed. She has over 20 years in bioinformatics and transcriptional profiling. She will be available on an as needed basis to help with analyzing single scRNASeq results.

NON-PERSONNEL

Travel ($33,582): We request a budget of approximately $3,716/year for the cost of attendance at a national/international meeting on sexually transmitted infections and T cell immunity that will be a showcase for description of the studies described in this proposal. This travel estimate includes conference registration fees, coach airfare on a domestic U.S. flag carrier, ground transportation, three nights lodging and four days per diem. Travel costs were estimated based on historical costs for past trips by faculty and students to conferences outside of California. A total of $ 18,582 is requested.

An additional $3,000 per year is budgeted to the cover the costs for Dr. Rafi Ahmed, Ph.D. to travel to UCI from Emory Vaccine Center to provide the consultation, presentation, and analysis of the project. A total of $15,000 is budget for Dr. Ahmed's travel.

OTHER DIRECT COST:

Supplies and Materials ($193,784): Reagents, disposable plastic, antibodies: The proposed studies will utilize a variety of fluorescently conjugated antibodies (approximately $8,000/year), real time PCR reagents for

6255

$540.00/year), reagents for magnetic sorting of cells, and extensive use of disposable plastic ware for performing in vitro immunologic assays, maintaining cell lines, performing injections, etc. (approximately $20,500/year). Use of Flow Cytometry: 16hrs/month @ $50/hr (approximately $7450/Year). Based on preliminary work we estimate the cost of these reagents and supplies to be approximately $38,757 each year over the five-year grant period.

Publication Costs ($10,618): Funds for publication-related expenses are requested in all years to cover the cost of manuscript fees, purchasing reprints, color figures, and poster costs associated with the dissemination of research results at national scientific conferences. These costs were estimated using the published reprint and page charges based on historical costs. An approximately $2,124 each year to defray cost related to publication of two manuscripts yearly, which is the average per grant for the last 5 years of our laboratory productivity.

Consultant Services ($15,000): Rafi Ahmed, Ph.D., a professor of immunology and the director at the Emory Vaccine Center will be actively involved in this project. We are requesting $3000/year to cover the cost of Dr. Ahmed's traveling to UCI each year for consulting, presentation, and analysis of the project.

Sub awards/Consortium/Contractual Costs ($163,800): Sunomix therapeutics will be responsible to deliver the gD, VP16, VP22 RR1 or RR2 proteins-based SAPNs to Dr. Lbachir BenMohamed Lab at UCI, California, to be used for the Herpes vaccine grant. As Sunomix Therapeutics, Dr. Burkhard and Dr. Bouziane will be directly involved in the design, development, cloning, protein expression, purification, refolding, TLR-activation and analysis of SAPN prototype to make them suitable as a platform for HSV vaccine. At least ten gD, VP16, VP22 RR1 or RR2 proteins-based SAPNs identified from herpes genome in Dr. BenMohamed lab will be produced at Sunomix Therapeutics. All the nanoparticles will contain CpG, the preliminary data using SAPNs produced by Sunomix Therapeutics shows very promising results.

Facility and Administrative Cost Rate (F& A): Facilities and Administrative costs were estimated in accordance with UCI's approved indirect cost rate agreement. UCI's indirect cost rate agreement approved by DHHS, the Federal Cognizant Audit Agency for UCI, on April 27, 2011 authorized the rate of 54.5% effective July 1, 2015. In the subject proposal, per policy, MTDC excludes graduate student tuition and fee remission.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		718,996.00
Section B, Other Personnel		719,382.00
Total Number Other Personnel	14	
Total Salary, Wages and Fringe Benefits (A+B)		1,438,378.00
Section C, Equipment		
Section D, Travel		33,582.00
1. Domestic	33,582.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		750,229.00
1. Materials and Supplies	193,784.00	
2. Publication Costs	10,618.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	163,800.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	382,027.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		2,222,189.00
Section H, Indirect Costs		1,135,447.00
Section I, Total Direct and Indirect Costs (G + H)		3,357,636.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		3,357,636.00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Peter		Burkhard		Consortium Principal Investigator	189,600.00	1.2			18,960.00	4,930.00	23,890.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **23,890.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	3			15,000.00	3,900.00	18,900.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Project Scientist	0.6			9,480.00	2,465.00	11,945.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**30,845.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**54,735.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT12838353
Funding Opportunity Number: PA-19-056 . Received Date: 2019-04-11T18:17:49.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	27,165.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**27,165.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**81,900.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**81,900.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	81,900.00

L. Budget Justification*	File Name: Budget_Justification_Sunomix1011294170.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Peter		Burkhard		Consortium Principal Investigator	189,600.00	1.2			18,960.00	4,930.00	23,890.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **23,890.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	3			15,000.00	3,900.00	18,900.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Project Scientist	0.6			9,480.00	2,465.00	11,945.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**30,845.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**54,735.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT12838353
Funding Opportunity Number: PA-19-056 . Received Date: 2019-04-11T18:17:49.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	27,165.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**27,165.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**81,900.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**81,900.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	81,900.00

L. Budget Justification*	File Name: Budget_Justification_Sunomix1011294170.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Peter		Burkhard		Consortium Principal Investigator		0.01			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*

Total Number Other Personnel **Total Other Personnel**

Total Salary, Wages and Fringe Benefits (A+B) **0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-04-11T18:17:49.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	0.00

L. Budget Justification*	File Name: Budget_Justification_Sunomix1011294170.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Peter		Burkhard		Consortium Principal Investigator		0.01			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-04-11T18:17:49.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	0.00

L. Budget Justification*	File Name: Budget_Justification_Sunomix1011294170.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Peter		Burkhard		Consortium Principal Investigator		0.01			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*

Total Number Other Personnel **Total Other Personnel**

Total Salary, Wages and Fringe Benefits (A+B) **0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-04-11T18:17:49.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 080437688

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Sunomix Therapeutics Inc

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	0.00

L. Budget Justification*	File Name: Budget_Justification_Sunomix1011294170.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Sunomix Therapeutics, San Diego, CA

Year 1: Direct Cost: $81,900, Indirect cost: 0; Total Cost: $81,900
Year 2: Direct Cost: $81,900, Indirect cost: 0; Total Cost: $81,900

PERSONNEL:

Peter Burkhard, Ph.D., Principal Investigator 1.2 Calendar Months $ 47,780

Dr. Burkhard will be directly involved in the design, development, cloning, protein expression, purification, refolding, TLR-activation and analysis of the gD, VP16, VP22 RR1 or RR2 proteins-based SAPNs. The prototypes will be send to Sunomix for scale up production. 10% salary at NIH cap with 26% benefit rate is budgeted for the PI.

Mohammed Bouziane, Ph. D, Project Scientist .60 Calendar Months $23,890

Dr. Bouziane have extensive experience in leading multi-disciplinary groups from Discovery, R&D to preclinical, and early clinical programs. Strong and broad scientific expertise covering multiple areas of genomics, proteomics, mutagenesis, nanoparticles SAPNs, diagnostics, vaccines and cell therapies for the treatment of infectious diseases, cancer and immunotherapies.

Dr. Bouziane will also assist the PI with the in vivo work and data analysis as described in this application. He will supervise a postdoctoral fellow. They will meet weekly to discuss results of SAPNs bio production. 5% salary at NIH cap with 26% benefit rate is budgeted for the PI.

Post-Doc Researcher 3.0 Calendar Months $37,800

We are requesting 6 calendar months' time and effort for this postdoctoral fellow. He will be responsible for the bio production of SAPNs under Dr. Bouziane supervision including: construction design, PCR, cloning, sequencing, protein expression, purification, refolding, TLR-activation and analysis of SAPN prototype to make them suitable as a platform for HSV vaccine. At least ten prototype that contain different pairs of CD4 and CD8 human epitopes identified from herpes genome in Dr. BenMohamed will be produced. He will also assist the CO-PI data analysis and working with Alpha-O-peptides. 25% of $60,000 salary with 26% benefit rate for the postdoc is budgeted.

MATERIALS AND SUPPLIES:

Lab Consumables, Molecular Biology, Gene synthesis-Cloning,
Sequencing of 10 SAPNs. We are requesting a total budget over 2 year duration of: $ 15,000

The DNA coding for the 10 nanoparticles SAPNs constructs will be prepared using standard molecular biology procedures. Plasmids containing the DNA coding for the protein sequence are constructed by cloning into the suitable (mostly NcoI, BamHI, NheI and EcoRI) restriction sites of the basic SAPN expression plasmid. This construct is composed of a pentameric coiled-coil tryptophane zipper linked by a glycine residues to a trimeric de-novo designed leucine coiled coil that for some constructs contains a panDR binding CD4+ epitope string. At the C-terminus the protein chain may be extended by a flagellin construct composed of the D0 and D1 domains of Salmonella enterica flagellin from the structure with pdb-code 3V47 from the RCSB protein data bank as in the prototype.

We need molecular biology Kits and reagents for PCR, cloning, lab consumables to support our in vitro experiments for our various vaccine constructs, as well as gloves, test kits including mycoplasma and endotoxin, safety supplies and other miscellaneous supplies necessary for a routine functional laboratory.
We are also asking for a yearly budget for the purchase of all of the required plastic ware that is normally used in the laboratory for PCR and molecular biology and virology applications. This includes flasks of all sizes, plates between 6 and 96 wells for PCR, disposable pipettes between 1 and 25 ml volume, barrier tips, and other

necessary plastics to carry out the experiments described in the proposal. This category will include other disposable items such as Eppendorf multi-channel tips, and all other liquid handling supplies.

Lab Consumables, Protein Expression, Large scale production of 10 SAPNs:
We are requesting a total budget over 2 year duration of: $15,000

The plasmids for 10 SAPNs are transformed into Escherichia coli BL21 (DE3) cells. Expression is induced with isopropyl β-D-thiogalacto-pyranoside. Alternatively, also other cell lines can be used for expression, such as tuner or KRX cells. Diluting the pre-cultures into the expression culture. The protein expression level is assessed polyacrylamide gel electrophoresis (SDS-PAGE).
We need bottles of tissue culture medium for scale up production of various vaccine constructs, as well as gloves, SDS-PAGE, test kits, safety supplies, gowns, sterile syringes and needles, and other miscellaneous supplies necessary for a routine functional laboratory. We are also asking for a yearly budget for the purchase of all of the required plastic ware that is normally used in the laboratory for in vitro maintenance of cell cultures and protein expression. This includes flasks of all sizes, disposable pipettes between 1 and 25 ml volume, purification flasks, barrier tips, and other necessary plastics to carry out the experiments described in the proposal. This category will include other disposable items such as Eppendorf multi-channel tips, and all other liquid handling supplies.

Lab Consumables, Protein Purification, Refolding of 10 SAPNs:
We are requesting a total budget over 2 year duration of: $10,000

Protein purification for 10 SAPNs constructs. the gD, VP16, VP22 RR1 or RR2 proteins-based SAPNs. We are also asking for a yearly budget for the purchase of all of the required protein purification and sequencing including: Ni-NTA Agarose Beads (Qiagen, Valencia, CA, USA); Purification columns ; Lysis, elution buffers; SDS-PAGE.

We are requesting a total budget over 2 year duration of: $14,330

Protein refolding: For refolding the protein is first rebuffered in specific buffer solution without urea. This quick dilution from denaturing (urea) to native (no urea) buffer conditions triggers refolding of the protein. The solution is then analyzed by negative stain transmission electron microscopy at different resolutions. If needed further screens for optimal refolding conditions can be performed with smaller sampling sizes of the pH and the ionic strength. Additionally, excipients such as trehalose, sucrose, arginine, proline or others can be added, or if needed detergents such as cholate, deoxycholate, tween-80 or others can be added.

TLR-activation. Activation through TLR5 will be assessed for different SAPN prototypes. The testing will be done using TLR/NF-kB/ SEAPorter™ Stably Transfected HEK 293 Stable Cell Lines as follows: All cell lines are stably co-transfected cell lines, which expresses the TLR5 and the secreted alkaline phosphatase (SEAP) reporter gene under the transcriptional control of an NF-kB response element. Using the 96-well plate format assays, TLR/NF-kB/SEAPorter™ HEK 293 cell line are used for screening of compounds as potential TLR5 agonists. The extent of SEAP secreted into the media is indicative of the amount of agonist activity. SEAP catalyzes the hydrolysis of p-Nitrophenyl phosphate (PNPP) producing a yellow product that can be read in a spectrophotometer or ELISA reader at 405 nm. Different concentrations of compounds are used to yield an EC50 value for each compound tested. Positive controls are made using native flagellin. Each compound will be tested in duplicate. Standard methodology for agonist testing is incubation of compounds in triplicate in 96 well plates at 5X104 cells/well.

Cells are stimulated with control ligand or test compounds at various concentrations. After 24 hour incubation SEAP is analyzed using SEAPorter™ Assay Kit. Dose-responsive percent activation of each sample well will be calculated to yield the ligand EC50 value.

Analysis of the biophysical properties of the SAPNs. The shape and size of the SAPNs will be analyzed using Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS).

We are requesting a budget for Sunomix over 2-year duration of $ 163,800

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		47,780.00
Section B, Other Personnel		61,690.00
Total Number Other Personnel	4	
Total Salary, Wages and Fringe Benefits (A+B)		109,470.00
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		54,330.00
1. Materials and Supplies	54,330.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		163,800.00
Section H, Indirect Costs		
Section I, Total Direct and Indirect Costs (G + H)		163,800.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		163,800.00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	473,908	483,552	411,385	421,488	431,856	**2,222,189**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

6255

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

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3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	SpecificAims1011294158.pdf
3. Research Strategy*	ResearchStrategy1011294159.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5 [redacted]	
6. Select Agent Research	BiohazardsHSV_1_and_HSV_21011294169.pdf
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	ConsortiumContractualArrangement1011294161.pdf
9. Letters of Support	SupportLetters_1011294163.pdf
10. Resource Sharing Plan(s)	ResourceSharingPlan1011294174.pdf
11. Authentication of Key Biological and/or Chemical Resources	AuthenticationKeyBioChemResources1011294165.pdf
Appendix	
12. Appendix	

A Prime/Pull Therapeutic Vaccine to Prevent Recurrent Genital Herpes

In the United States, over 60 million adults (15-49 years of age) and over 536 million worldwide bear herpes simplex virus type 2 (HSV-2) infection[1-4]. There is currently no licensed genital herpes therapeutic vaccine. Antiviral drug therapies ease genital herpes outbreaks and symptoms but neither cure the disease nor eliminate the virus, the roots of the disease. Prevalence of herpes infection is higher in women than in men[5]. Genital herpes is associated with severe medical and psychological consequences, a risk of virus transmission from women to neonates that could lead to severe neurologic disease or death in the newborn and an increased risk of HIV acquisition. Vaginal mucocutaneous surfaces (abbreviated as VMC) serve as the entry point for HSV-2, leading to the establishment of a lifelong latent infection in the sensory neurons of the lumbosacral dorsal root ganglia (abbreviated as DRG)[6]. Following sporadic reactivations of the virus from latency in the DRG, the virus travels back in the axons to re-infect the VMC tissue[7] (see lifecycle of HSV-2 in **Fig. 1** below). Recurrent genital herpes disease is then caused by virus replication of the VMC epithelial tissues.

Our long-term goal is to develop a therapeutic vaccine to protect against recurrent genital herpes. Over the last 25 years, several types of HSV-2 vaccines have been attempted, eliciting mostly strong systemic antibody responses. However, in clinical trials, they failed to protect therapeutically or prophylactically. The DRG and VMC tissues are the most obvious battlefield sites for host's B- and T-cells to control recurrent HSV-2. Besides antibodies, HSV-specific $CD8^+$ T cells and $CD4^+$ T cells are critical in preventing or aborting virus reactivations from latently infected neurons of DRG (designated in this proposal as central neuronal immunity)[8-16]. $CD4^+$ and $CD8^+$ T cells also limit virus replication in the VMC epithelium (designated in this proposal as peripheral epithelial immunity) [3, 4, 17, 18]. Preclinical studies in the HSV-2 infected 6255 model and observations in HSV-2 infected asymptomatic women without genital herpes indicate the presence of tissue-resident virus-specific $CD4^+$ and $CD8^+$ T cells at the healed sites of the VMC epithelia (Ref [1] and **Figs. 2** to **12**).

During the last 5 years, we have made significant progress in identifying candidate HSV-2 antigens and characterizing the phenotype and function of antiviral circulating and tissue-resident T cells that are associated with protection in seropositive women and in the established 6255 model of recurrent genital herpes: (**A**) We found that two HSV-2 tegument virion proteins (VP16 and VP22) and two ribonucleotide reductase subunit proteins (RR1 and RR2) are mainly targeted by $CD4^+$ and $CD8^+$ T cells from "naturally" protected asymptomatic women (those who, despite being infected, never develop recurrent genital herpes)[1]; (**B**) Similarly, VP16, VP22, RR1 and RR2 proteins were the main HSV-2 antigens recognized by tissue-resident $CD4^+$ and $CD8^+$ T cells that reside in the DRG and VMC sites of protected asymptomatic 6255 (ref[1] and **Fig. 3**); (**C**) Phenotypic and transcriptomics RNA-Seq profiling indicate that, similar to HSV-2 VP16-specific $CD8^+$ T cells in HSV-2 infected asymptomatic women (**Fig. 4A**), $CD8^+$ T cells from healed VMC-resident $CD8^+$ T cells in protected 6255 bear all the hallmarks of functional tissue-resident $CXCR3^+CD8^+$ T cells (**Figs. 4B-4E**); (**D**) While therapeutic vaccination with RR2 antigen produced strong protection in HSV-2 infected 6255 the VP16, VP22, and RR1 antigens provided modest protection[1]; (**E**) Similar to humans (**Fig. 4A**), RNA-Seq data indicate that $CD4^+$ and $CD8^+$ T cells in the DRG and VMC of protected 6255 expressed high levels of *CXCR3* gene (**Fig. 4B**); and (**F**) Treating HSV-2 infected 6255 with a neurotropic adeno-associated virus (AAV8) vector expressing the 6255 CXCL11 chemokine (a CXCR3 ligand) boosted the number of $CD4^+$ and $CD8^+$ T cells specifically in infected DRG and VMC and improved protection against recurrent genital herpes (**Figs. 5, 6**, **10**, **11** and **12**). Building on the above strong published[1, 19-23] and preliminary data, **we hypothesize** that a therapeutic vaccine that boosts potent antiviral tissue-resident $CD4^+$ and $CD8^+$ T cell responses locally in DRG and VMC would produce a more robust/sustained protection against HSV-2 reactivation and shedding and reduce recurrent genital herpes. To test this hypothesis, we propose the following two **Specific Aims**:

Aim 1: To evaluate the safety and protective efficacy, in the 6255 genital herpes model, of an innovative prime/pull therapeutic vaccine approach that consists of: (**1**) Priming T cells with VP16, VP22, RR1, and RR2 antigens, using self-assembling protein nanoparticles (SAPNs) as a delivery system; and (**2**) "Pulling" primed T cells into infected DRG and VMC tissues by a local delivery of 6255 T-cell attracting chemokines, CXCL9, CXCL10 and/or CXCL11, using a neurotropic AAV8 vector.

Aim 2: To determine whether increasing the number and function of antiviral tissue-resident $CD4^+$ and $CD8^+$ T cells within: (1) DRG (central neuronal immunity); and (2) VMC (peripheral epithelial immunity) will correlate with protection against recurrent genital herpes infection and disease.

This pre-clinical translational vaccine research, that involves a multidisciplinary team of six scientists with complementary expertise, is expected to lay the foundation for developing a T-cell-based prime/pull therapeutic vaccine to protect from recurrent genital herpes. The project is highly relevant to NIAID's current mission of developing fundamental knowledge that may reduce the burden of human disease .

6255

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SIGNIFICANCE

1. *Most genital herpes is due to reactivated HSV-2 from latently infected DRG, rather than to primary HSV-2 infection:* Despite several efforts, a safe and efficient genital herpes vaccine is still not available. Virus shedding and re-infection of the VMC may be either (**1**) Asymptomatic (ASYMP), with mild and unrecognized disease[24]; or (**2**) Symptomatic (SYMP), with severe and painful mucocutaneous genital lesions leading to complications including urinary retention and a substantial psychological illness[25, 26]. Lifecycle of genital HSV-2 infection is shown in **Fig. 1** below. Despite widely used methodologies to control genital herpes, including antiviral drugs (Acyclovir and derivatives), education, and the use of condoms, the spread of genital herpes infection remains an epidemic in some populations[27] [28, 29]. A body of evidence suggests that only the widespread use of an effective vaccine can prevent or reduce symptomatic disease and eliminate or at least limit asymptomatic viral shedding, which may in turn help control the herpes simplex epidemic [28-31].

2. *Lessons learned from past genital herpes vaccine clinical trials:* Four main vaccine approaches have been tested in the past four decades to fight herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) infections and diseases [32]: (**1**) **Inactivated "killed" HSV vaccines**; (**2**) **Live-attenuated HSV vaccines**; (**3**) **Replication-defective HSV vaccines**; and (**4**) **Subunit HSV vaccines**. Back in the 70s and 80s, the first whole inactivated HSV vaccine approach used "killed" virus after exposure to heat, UV light[33] or chemicals[34, 35]. While inactivated HSV vaccines induce antibodies, they fail to induce robust T cells, and as such have not been successful in protecting against recurrent genital herpes[36-38]. Therefore, live-attenuated HSV vaccines[39-44] and replication-defective HSV vaccines were later introduced[36-38, 45-50]. Due to weak immunogenicity and safety concerns, few replication-defective and live-attenuated vaccines have progressed into clinical trials[51]. Live-attenuated and replication-defective HSV vaccines carry the risk of regaining their pathogenicity under immunocompromised conditions[51]. *To avoid safety issues, which may occur with live-attenuated and replication-defective vaccines, protein-based subunit vaccines have been attempted.* Recombinant soluble HSV-2 glycoprotein D (gD) has been the most subunit vaccine in extensive clinical trials over the past 25 years[52-56]. In 1994, the first therapeutic vaccine trial used gD with aluminum salt (i.e., Alum) adjuvant and reduced recurrence frequency by only 24%, despite that the vaccine boosted virus neutralizing antibodies[57]. In 1997, the Chiron vaccine trial used a combination of gD and gB delivered with Novartis's MF59 adjuvant, an oil-in-water emulsion of squalene oil[52]. This gB/gD/MF59 vaccine produced high levels of neutralizing antibody yet had only 9% efficacy[52]. Later, two GlaxoSmithKline (GSK) vaccine trials (one reported in 2004[53] and the other in 2012[58]) used the gD protein delivered with a different adjuvant, 3-*O*-deacylated monophosphoryl lipid A (MPL), a TLR4 agonist together with Alum (Herpevac Trial for Women). The main goal was to protect women against genital herpes disease. The first trial enrolled discordant couples who had regular partners with genital herpes, while the second trial enrolled HSV seronegative women who had multiple and random partners[53]. The first trial had 74% efficacy against genital herpes disease caused by HSV-2[53]. Unfortunately, the second trial, which used the same gD/MPL/Alum vaccine, showed only 58% efficacy against genital HSV-2 disease[58]. The apparent inconsistencies in protection from these two clinical trials were attributed to different populations enrolled in each trial (i.e., discordant couples vs. random seropositive women with multiple partners)[58]. In 2016-2018, a Genocea vaccine trial (Gen-003) combined gD and ICP4 truncated proteins with a novel adjuvant, Matrix M-2 (MM-2)[59]. This trial reported a reduction of recurrent herpes lesions and genital viral shedding, but was recently terminated[54-56]. This protection appeared to correlate with blood-derived antiviral $CD4^+$ and $CD8^+$ T cell responses[54-56]. However, due to ethical and practical limitations, none of these vaccine clinical trials investigated the role of local tissue-resident $CD4^+$ and $CD8^+$ T cells in DRG and VMC tissues in protection. The above clinical vaccine trials emphasize **two major gaps in our current knowledge**: (**1**) The need to incorporate protective herpes Ags, other than gB and gD proteins, in a future herpes vaccine[60]; and (**2**) The need to design a vaccine strategy that induces antiviral $CD4^+$ and $CD8^+$ T cell-mediated immunity (in addition to HSV-specific neutralizing antibodies) [60].

Figure 1. HSV-2 life cycle is similar in humans and the 6255 **model.** ❶ Primary infection. ❷ Virus to latency in DRG. ❸ Virus reactivation from DRG. ❹ Re-infection of, and virus shedding from VMC.

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We strongly feel that the appropriate response to the above "failures" of HSV vaccine clinical trials using one or two HSV glycoproteins with adjuvant, is to increase our efforts and not to throw our hands into the air and give up. In this vein, there is much work to do to increase our understanding of HSV immunology and to test novel antigen delivery approaches in 6255 such as the 6255 that may prove to be more efficacious in man.

3. *Antigen selection and self-assembling protein nanoparticles (SAPNs) delivery system that targets both endogeous (MHC-I) and exogenous (MHC-II) pathways:* A proteomic library containing a near-complete collection of the HSV-2 84$^+$ open reading frames (ORFome) of HSV-2 has been cloned and corresponding proteins expressed individually[61, 62]. HSV-2 protein antigens recognized by T cells from "naturally protected" seropositive individuals resistant to recurrent genital herpetic disease despite being infected—were selected as antigen targets for designing a protein-based subunit herpes prime/pull vaccine for this project. As detailed in our recent publication in *The Journal of Virology*[1], a set of four HSV-2 Ags, VP16, VP22, RR1 and RR2 tegument and regulatory proteins, were recognized mostly by CD4$^+$ and CD8$^+$ T cells from "naturally protected" ASYMP individuals[1]. Moreover, VP16, VP22, RR1 and RR2 based vaccines induced both HSV-2 neutralizing antibodies and antiviral vaginal mucosa-resident CD4$^+$ and CD8$^+$ T cells[1]. (US Provisional Patent Application US.608096 has been filed). Spherical self-assembling protein nanoparticle (designated as SAPNs)-Ag-based vaccines have been reported to induce protective antibody CD4$^+$ and CD8$^+$ T-cell responses in other systems[63-73]. Therefore, we selected the SAPNs delivery system as it presents protein antigens to both exogenous (MHC-II) and endogeous (MHC-I) pathways, *via* a cross-presentation mechanism[63-73]. We will use these nanoparticle-based vaccines to better present VP16, VP22, RR1, and RR2 protein antigens to the immune system *in vivo*. In these nanoparticles, a repetitive antigen display technology relies on the self-assembly of 60 protein chains into SAPNs, which will protect these proteins from premature proteolytic degradation, and facilitate their uptake and processing by antigen presenting cells. In addition, SAPN technology is designed to eliminate toxicity and safety issues associated with the use of external immuno-adjuvants by incorporating portions of the TLR5 agonist flagellin, as a built-in immuno-adjuvant. By combining different molar ratios of VP16, VP22, RR1, and RR2 into portions of flagellin monomers before self-assembly, we generated multiple nanoparticles and investigated their biophysical characteristics. Thus, our studies already assembled the VP16, VP22, RR1, RR2 and gD proteins either as single HSV-2 protein together with flagellin (example of SAPN-VP22 nanoparticles shown in **Fig. 2A**, *left panel* and **Fig. 7A**) or in combination of 2 different HSV-2 proteins together with flagellin (example of SAPN-VP16/VP22 nanoparticle shown in **Fig. 2A**, *right panel*). In a pilot study, we demonstrated that subcutaneous injection of a prototype SAPN vaccine induced significant IFNγCD8$^+$ T-cell responses (**Fig. 2B**). This result confirms and extends previous reports that SAPN protein-based vaccines induced protective CD4$^+$ and CD8$^+$ T-cell responses in other systems[63-73].

Figure 2A. Examples of SAPN-VP22 protein and SAPN-VP22/VP16 combination of two different proteins. Monomers are on *top* and particles at the *bottom*.

Figure 2B. Sub-cutaneous injection of *SAPN-Ag based vaccine induced antiviral IFNγ$^+$CD8$^+$ T cells.*

4. *Established 6255 model of genital herpes for preclinical testing of therapeutic vaccine candidates:* A major hindrance to research in developing a therapeutic herpes simplex vaccine has been the selection of a suitable 6255 model. For most herpes immunologists, 6255 (e.g., C57BL/6 and BALB/c strains) is the preferred model system to study the immunology of genital herpes following primary infection[74]. Yet recurrent virus shedding in the vaginal tract and recurrent herpetic genital disease does not occur in mice[75]. Therefore, although 6255 studies have provided important information regarding genital HSV-2 infection and immunity[76-80], the efficacy of vaccine candidates against recurrent genital herpes shedding and disease cannot be assessed in mice. The success of preclinical vaccines that show protection in 6255 models of HSV-2 intravaginal challenge has not been successfully translated into clinical trials. The 6255 model is the gold standard small 6255 model that mimics spontaneous recurrent genital herpes as it occurs in humans.

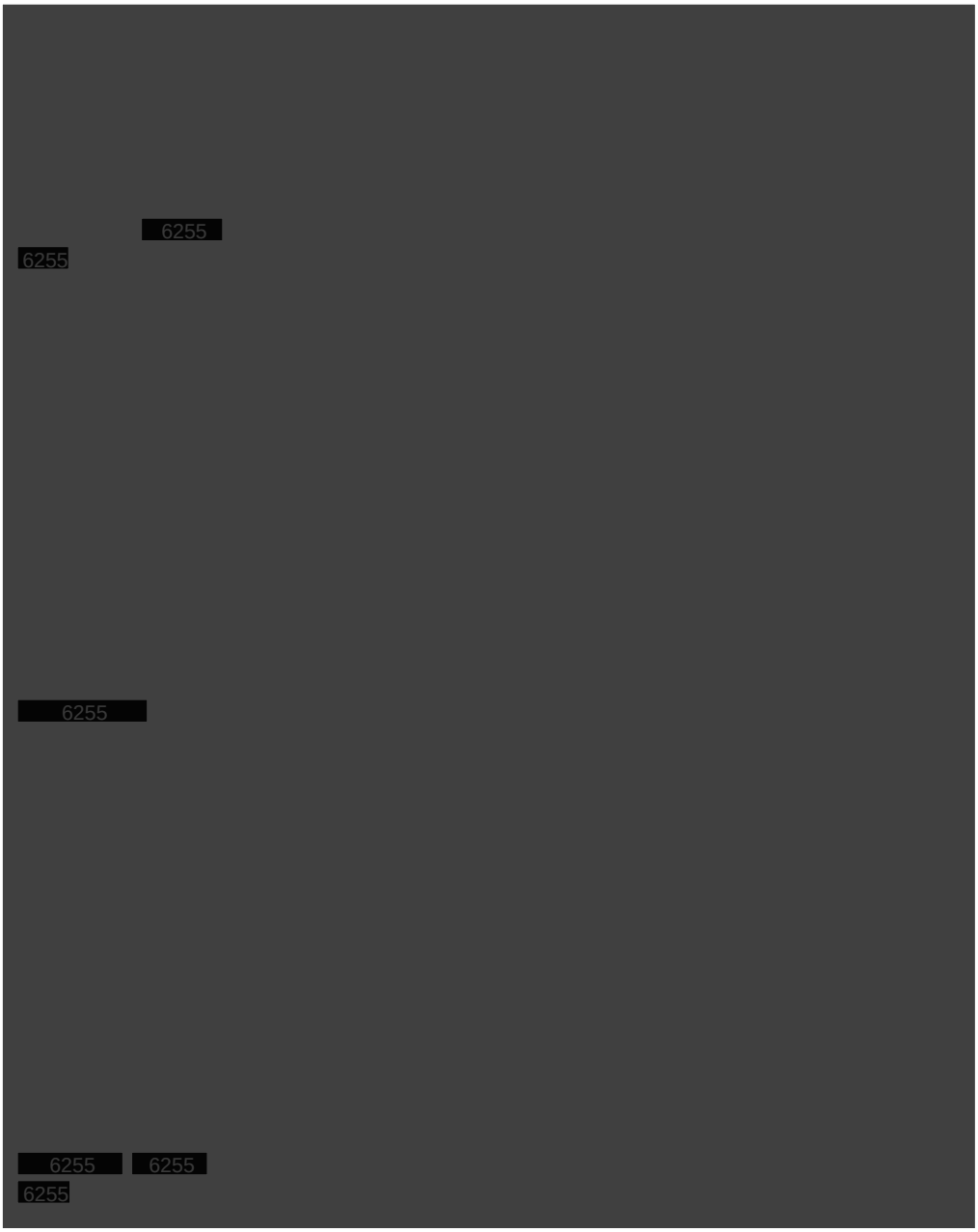
Figure 3. Increase numbers of CD4$^+$ and CD8$^+$ T cells localized to DRG tissues of protected guinea (*top*) compared to DRG of non-protected 6255 (*bottom*).

5. *Unprecedented immunological assays are now possible in the 6255 model:* For over 3 decades, it has not been technically feasible to perform phenotypic, functional, and transcriptional profiling of

memory CD4+ and CD8+ T cell subsets in the 6255 model. One major limitation was the unavailability of monoclonal antibodies (mAbs) specific to 6255 T cell subsets, surface CD, cytokines, and chemokines. In the last 5 years, we have advanced T cell immunology frontiers in the 6255 model. In collaboration with Dr. Hubert Schäfer (see letter), we have developed cutting-edge assays for phenotypic (**Figs. 3** to **12**) and functional (**Figs. 4** and **11**) characterization of circulating and tissue-resident CD4+ and CD8+ T cells of the 6255 6255 model of genital herpes. We now have developed a panel of new mAbs and immunological assays together with single-cell scRNA-Seq specific to 6255 effector, regulatory, and memory CD4+ and CD8+ T cells, allowing unprecedented opportunities to assess the phenotype and function CD4+ and CD8+ T cells within the DRG and VMC after vaccination of HSV-2-infected 6255 Functional T cell assays, including cells IFN-γ-ELISpot, CFSE-based proliferation, surface markers of T cell activation (CD25, CD44, CD69 and CRTAM) and T cell exhaustion (PD-1, LAG-3 and TIM-3), intracellular cytokines can now all be assessed in the 6255 model [(1)]. Recently, we successfully performed transcriptomic RNA-Seq profiling of 6255 CD8+ T_{RM} cells sorted from HSV-2-infected DRG (**Fig. 4B**) and VMC (*data not shown*).

6. The CXCL9, CXCL10, CXCL11/CXCR3 axis for T cell activation: A target for a novel prime/pull therapeutic herpes vaccine:

Chemokines are proteins that induce chemotaxis, promote T cell differentiation, and cause tissue extravasation[(85)]. CXCL-9, -10, and -11 are mainly secreted by monocytes, endothelial cells, and fibroblasts[(86)]. Dr. Carr reported in 2008 that CXCL9 and CXCL10 expression are critical for control of genital HSV-2 infection while CXCR3 deficiency increases susceptibility to HSV-2 infection[(87-89)]. Later in 2012, Dr. Iwasaki reported that intravaginal administration of CXCL10 chemokine in non-infected immunized mice attracted T cells to VMC and protect from HSV-2 challenge[(90)]. Using several molecular biology and immunological assays, we discovered that the CXCL9, CXCL10, CXCL11/CXCR3 axis is highly activated in DRG and healed VMC of HSV-2 infected and protected 6255 (i.e., asymptomatic) compared to non-protected 6255 that present severe genital herpes symptoms (**Figs. 4B** to **4E**). The RNA-Seq preliminary results revealed that similar to HSV-2 VP16-specific CD8+ T cells in asymptomatic humans (**Fig. 4A**), elevated levels of the *CXCR3* receptor gene in tissue-resident CD8+ T cells FACS-sorted from DRG of HSV-2 infected and protected vs. non-protected 6255 (**Fig. 4B**). In contrast, RNA-Seq showed a modest upregulation of *CCR5* and *CCR10* receptor genes in T cells from protected compared to non-protected 6255 (*data not shown*). Moreover, phenotypic profiling



of latently healed VMC-resident T cells in protected 6255 showed frequent $CXCR3^+CD4^+$ and $CXCR3^+CD8^+$ T cells, compared to non-protected 6255 (**Fig. 4C**). Western blot and immunostaining confirmed significant expression of the CXCL9, CXCL10, CXCL11 chemokines in healed VMC of HSV-2 infected and protected 6255 (**Fig. 4D** and **4E**) suggesting that the CXCL9, CXCL10, CXCL11/CXCR3 axis is involved with a yet-to-be-determined mechanism in activating antiviral tissue-resident $CD4^+$ and $CD8^+$ T cell responses locally in DRG and VMC. We hypothesize that a successful HSV therapeutic vaccine would have to attract large numbers of protective T cells to the affected DRG and VMC.

7. *Identifying the neurotropic adeno-associated virus serotype 8 (AAV8) as a vector for increasing numbers of protective T cell in the 6255 DRG and VMC:*

AAV vectors are useful vehicles for delivering transgenes to infected tissues and organs *in vivo*[91-93]. Recently, there has been much progress in delivering AAV vectors to sensory neurons following application to the peripheral epithelium, such as skin or eye[91-93]. This delivery requires scratching the epithelium to access the underlying nerve termini. We compared AAV1-GFP, AAV8-GFP, and AAV9-GFP vectors in HSV-2 infected 6255 and discovered the AAV8-GFP vector's superiority at 8 days after footpads inoculation (**Fig. 5A**). Compared to intravaginal, a better transduction in DRG and VMC was obtained following footpad inoculation (*data not shown*). Moreover, a neurotropic AAV8 expressing GFP and the 6255 T cell-attracting CXCL11 chemokine, both under the neurotropic human synapsin 1 gene promoter (hSYN) (AAV8-hSYN-eGFP-hSYN-gpCXCL11 vector) (**Fig. 5B**), resulted in specific transduction of 6255 DRG and VMC tissues. Moreover, the AAV8 vector expressing the 6255 CXCL11 chemokine (a CXCR3 ligand) specifically in infected DRG tissue reduced recurrent genital herpes lesions (**Fig. 6**, *bottom left panel)* associated with increased the number of antiviral tissue-resident $CD4^+$ and $CD8^+$ T_{RM} and T_{EM} cells (**Fig. 6**, *bottom right panels)*.

Figure 5A. Successful delivery and expression of GFP in the DRG and VMC of HSV-2 infected 6255 after footpad injection of AAV8 vector, compared to AAV1 and AAV9 vectors. Representative data (*Left panel*). Average in 5 6255 (*right 2 panels*). (*) and (**) < 0.05.

Figure 5B. A prototype of the neurotropic AAV8 expressing the green fluorescent protein (GFP) and the 6255 gpCXCL11 under the neurotropic human synapsin 1 promoter (hSYN). We used ITR from AAV2 and AAV8 capsid for packaging.

INNOVATION

Conceptual innovation: Bolstering antiviral tissue-resident $CD4^+$ and $CD8^+$ T cell responses locally in HSV-infected DRG and VMC by stimulating the CXCL-9, -10, -11/CXCR3 axis is innovative. Exploiting the CXCL-9, -10, -11/CXCR3 axis in a prime/pull therapeutic vaccine to improve the immunogenicity of HSV-2 protein antigens is conceptually innovative. This is expected to produce robust and sustained protection against recurrent genital herpes. This novel vaccine strategy would constitute a paradigm shift in the genital herpes vaccine field. Preliminary data showing a frequency boost in $CXCR3^+CD4^+$ and $CXCR3^+CD8^+$ T_{RM} cells in DRG and VMC following treatment with a neurotropic AAV8 vector expressing the 6255 CXCL11 chemokine is innovative. The SAPN antigen delivery system which cross-present antigens to $CD4^+$ and $CD8^+$ T cells is innovative. Comparing the relative contributions of central neuronal immunity in DRG and of peripheral epithelial immunity in VMC in protection against recurrent genital herpes is innovative. Studying the role of SSG-resident $CD4^+$ and $CD8^+$ T cells in protection is innovative. Last, combining the prime/pull vaccine with PD-1 and LAG-3 immune checkpoint blockade to reverse potential exhaustion of antiviral $CD4^+$ and $CD8^+$ T_{RM} cells is innovative.

Technical innovation: We have developed and optimized unprecedented techniques for phenotypic, functional, and transcriptome characterization of $CD4^+$ and $CD8^+$ T cell subsets in the 6255 model[1]. The SAPNs antigen delivery system used in this proposal is innovative. We have also identified the neurotropic AAV8 serotype, among many other AAV serotypes, as an efficient vector to deliver 6255 T-cell-attracting chemokines (e.g., CXCL11) specifically to DRG, the site of latent herpes infection. Preliminary data showing decreased virus reactivation *ex vivo* in 6255 DRG explants following PDL1 and LAG-3 immune checkpoint blockade is innovative. The technical innovation is also reflected in our unprecedented ability to develop RNA-Seq in the 6255 and to develop in-house computational expertise necessary to analyze and interpret the

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large datasets resulting from the proposed single-cell scRNA-Seq experiments. These innovations are expected to advance the frontiers of T cell immunology in the 6255 genital herpes model over the next 5 years.

***Note for clarity**: Due to the ethical and practical limitations in obtaining DRG samples and biopsies from HSV-2-infected women, the characterization of $CD4^+$ and $CD8^+$ T cells was limited to blood- and VMC-derived T cells[81-84]. The 6255 model offers the possibility to study longitudinally the phenotype, function, transcriptome and specificity of both DRG- and VMC- resident T cells after infection and therapeutic vaccination.*

INVESTIGATORS

The Principal Investigator has gathered a multidisciplinary team that includes five top basic scientists in the following fields (all at UCI): (**1**) Microscopy (Dr. James V. Jester), (**2**) Mathematical modeling (Dr. Elizabeth Read), (**3**) Biostatistics (Dr. Christine McLaren), (**4**) Single-cell transcriptional profiling specialist (Dr. Melanie Oakes), and (**5**) Bioinformaticist (Dr. Jenny Wu). This multidisciplinary team of leading researchers will fundamentally improve existing concepts of genital herpes infection and immunity in the 6255 model.

OVERALL IMPACT

We expect this translational research project to produce a lead prime/pull therapeutic vaccine that has a durable protective clinical impact on recurrent genital herpes. This pre-clinical study using the 6255 model would be a critical first step toward developing a prime/pull therapeutic vaccine to prevent recurrent genital herpes in the clinic. In addition, this project is expected produce a new body of information regarding the role of central neuronal immunity (in DRG) and peripheral epithelial immunity (in VMC) in protection against genital herpes.

APPROACH

Specific Aim 1: To evaluate the safety and protective efficacy, in the [redacted] genital herpes model, of an innovative prime/pull therapeutic vaccine approach that consists of: (1) Priming T cells with SAPN particles presenting VP16, VP22 and RR antigens and; (2) "Pulling" primed T cells into infected DRG and VMC tissues using the neurotropic AAV8 vector expressing the [redacted] T-cell attracting CXCL9, CXCL10 and/or CXCL11 chemokines.

Rationale for using the [redacted] model of recurrent genital herpes:

Mice do not develop virus shedding or recurrent genital herpetic disease following intravaginal HSV-2 infection,[75] because virus reactivation from latently infected sensory neurons is extremely rare or does not occu[redacted] [75]. Thus, mice cannot be used to test therapeutic vaccine candidates. Moreover, vaccination approaches found valuable [redacted] have so far translated poorly, or not at all, to human (Reviewed in [74]). In contrast, the HSV-2-infected [redacted] fully recapitulates human spontaneous shedding leading to recurrent genital herpes. Although state-of-the-art [redacted] *immunology* still lags behind that of [redacted] *immunology*, during the past 5 years, in collaboration with Dr. Hubert Schäfer (see letter), we have advanced the frontiers of [redacted] *immunology* (Ref [1] and **Figs. 3** to **12**). The frequency, function and exhaustion of [redacted] [redacted] $CD4^+$ and $CD8^+$ T_{EM}, T_{RM}, and T_{CM} cell populations can now be easily characterized (**Figs. 6** and **11**).

[redacted]

Rationale for the prime/pull therapeutic vaccine: Our recent published therapeutic vaccine study in [redacted] indicates that increased number and function of DRG- and VMC-resident $CD4^+$ and $CD8^+$ T cells was associated with reduced severity and rate of recurrent genital herpes (Ref[1] **Figs. 3**, **4, 6**, **10, 11** and **12**). Initial Iwasaki observations indicate that intravaginal administration of CXCL10 chemokine in non-infected immunized mice attracted T cells to VMC associated with control of HSV-2 challenge[90]. However, no study has reported the effect of expression of T cell attracting chemokines on DRG-resident T cells and how that affects recurrent infection and disease. Our preliminary data indicate that intravaginal inoculation of HSV-2-infected [redacted] with a neurotropic AAV8 vector expressing the [redacted] CXCL11 chemokine under the neurotropic hSYN promoter (AAV8-hSYN-eGFP-hSYN- gpCXCL11 vector) (**Fig. 5B**), increased the pool of DRG- as well as of VMC-resident $CD4^+$ and $CD8^+$ T cells (**Fig. 6** *right panel*, and **Figs. 10, 11, 12**). This was associated with a significant decrease recurrent genital herpes disease (**Fig. 6**, *left panel*). These preliminary data support our hypothesis that a successful HSV therapeutic vaccine would have to attract large numbers of protective T cells to the affected DRG and VMC.

***Note for clarity**: Since most genital herpes disease is due to virus reactivation from latency, rather than to primary infection, this Aim focuses on a therapeutic vaccine that will prevent recurrent genital herpes. Many HSV-2-infected individuals undergo frequent viral reactivation (up to 40% of all days showed positive reactivated virus)[96]. Thus, a therapeutic herpes vaccine should not only cure symptomatic clinical recurrences, but also control asymptomatic viral shedding; thereby decreasing the virus's spread to sexual partners and newborns.*

Experimental Design: ***Establishing HSV-2-infected [redacted] with recurrent genital herpes:*** The [redacted] model of spontaneous recurrent genital herpes is currently being used in our lab ([1] and **Figs. 3** to **12**). Throughout this project, we will use the virulent MS strain of HSV-2, generously gifted by Dr. David Bernstein (University of Cincinnati, OH). Guinea pigs will be anesthetized, placed on their backs, and inoculated intravaginally (IVAG) with 25 µl of HSV-2 at a dose of 5 x 10^5 PFU per [redacted] [3]. Following intravaginal infection with the MS strain of HSV-2, ~10% of [redacted] develop severe recurrent genital herpes disease (SYMP [redacted] The remaining ~90% are infected, but do not develop any recurrent genital herpetic disease (ASYMP [redacted] To achieve a power of at least 0.90, a total of 20 [redacted] is required per group assuming that 10% of the total [redacted] will develop severe recurrent genital herpes disease. ***Prime/Pull therapeutic vaccination:*** On day 18 post-infection, latently infected [redacted] (*n* = 20) will receive: (**a**) Sub-cutaneous (SC)

injection of 50 µg of SAPNs particles expressing HSV-2 antigens (i.e., VP16, VP22, RR1, RR2, or gD protein (as shown in **Figs. 2A** and **7A**); and (**b**) on day 25 post-infection foot-pad (FP) inoculation with 5 x 10^6 pfu of AAV8 vector expressing T-cell attracting chemokines, CXCL9, CXCl10 and/or CXCL11 (**Fig. 7B**). We chose footpad inoculation of AAV8, instead on intravaginal administration, because this route showed better transduction of DRG and VMC (**Fig. 5A**). We selected the SAPNs delivery stem as it presents protein antigens to both exogenous (MHC-II) and endogeous (MHC-I) pathways, *via* a cross-presentation mechanism[63-73]. To construct these SAPNs, we leveraged our long-term partnerships with Sunomix Therapeutics, Inc., and Dr. Peter Burkhard, the inventor of this nanoparticle technology (patents US8575110, US8546337, EP2766386A1 and EP2766386A1), an active collaborator on this project (see letter).

Figure 7A. (A) SAPN-VP22 vaccine particle. Green is 60 copies of VP22 protein. Blue is flagellin adjuvant. (**B**) Transmission electron microscopy of a nanoparticle preparation.

We selected the neurotropic AAV8 vector because: (***i***) It is safe. Clinical trials using AAV vectors in gene therapies have shown only mild and transient inflammation while demonstrating clinical benefits[97]; (***ii***) It is efficient and specific in delivering chemokines to sensory neurons of the DRG[98-100]. We found that AAV8-GFP persistently expressed GFP both in the cell body of neurons (DRG) and in the axon termini (VMC) of [redacted] latently infected with HSV-2 (**Fig. 5A**); and (***iii***) AAV8 vector can accommodate up to 4.7 kb of DNA expressing multiple chemokines. Thirty-two different groups of HSV-2 infected [redacted] (*n* = 20/group) will be enrolled in this preclinical vaccine trial as shown in **Table 1**. We will then compare virologic and clinical impact in [redacted] that receive the prime/pull vaccine (*n* = 20) to the replication-defective HSV-2 *dl*5-29 mutant virus (i.e., *dl*5-29 vaccine, kindly provided by Dr. Knipe) and used as a positive control (*n* = 20). ***Safety:*** To ensure the safety, the group of [redacted] that received the lead vaccine out of 18 candidates (*n* = 20), will be monitored up to 12 months post-immunization for potential short-term local reactogenicity (e.g., swelling, inflammation, and redness at injection site) and long-term unwanted systemic side effects (e.g., loss of food consumption and reduction of body weight). Any undesirable side effect will be recorded and followed by histopathological examinations of pivotal organs (e.g., brain, sensory ganglia, and VMC). ***Monitoring genital herpes infection and disease:*** The primary endpoint is to prevent HSV-2 shedding and replication in the VMC. The secondary endpoint is the prevention of recurrent genital herpetic lesions caused by HSV-2 replication in the VMC tissues. These two parameters will be followed for 20, 30 and 60-days post-vaccination, as illustrated in **Fig. 7B**. The rate of viral shedding immediately after vaccination will be compared to baseline shedding rates before vaccination as well as to the rate of mock-vaccinated controls[54]. Genital herpes disease and virus shedding will be monitored daily by investigators blinded to [redacted] vaccination and treatment and will be scored according to a standard 0-4 scale, as we described[1]. Mean disease score measured daily for 60-days post-vaccination and cumulative mean lesion severity scores will be determined, as we described[1]. The number of days with recurrent lesions will also be determined in vaccinated and control groups[1]. ***Latency in DRG:*** A third endpoint will assess the amount of latency in DRG, of vaccinated vs. mock-vaccinated groups, as we previously reported[3]. DRG are harvested and the expression of immediate early, early, and late genes will be quantified by droplet digital RT-PCR (ddRT-PCR), to determine evidence of virus latency in DRG[3, 6]. Using IHC, will also determine whether tissue-resident $CD4^+$ and $CD8^+$ T cells are co-localized around sensory neurons in DRG. ***Latency in SSG:*** Evidence of HSV-2 reactivation in the autonomic sacral sympathetic ganglia (SSG) was recently reported by Dr. Krause's group[6]. Besides latency in DRG, we will determine latency in autonomic SSG neurons of vaccinated vs. mock-vaccinated groups. We will also determine whether tissue-resident $CD4^+$ and $CD8^+$ T cells are co-localized around autonomic neurons in SSG. ***Longevity of protection:*** We will determine whether protection induced by the lead prime/pull vaccine will be long-lasting in the vaccinated [redacted] (i.e., 12 months after vaccination). ***Ex vivo reactivation in explanted DRG:*** A separate experiment will use latently infected prime/pull vaccinated (*n* =20) and mock-vaccinated (*n* =

20) [redacted] to assess the vaccine's effect on viral reactivation, *ex vivo*, in explanted sensory ganglia as shown in **Fig. 8**. Seven and 21 days after immunization, DRG will be harvested and individually explanted into tissue culture media to permit detection of reactivating virus, as described[6, 101]. Immediate early, early, and late gene expression will be quantified by droplet digital RT-PCR (ddRT-PCR) for evidence of viral reactivation in the explanted DRG, as recently described[6]. The time course of first appearance of the reactivated virus will be determined[101]. The % of DRG that reactivate and the kinetics of *ex vivo* reactivation will be compared in the vaccine vs. control groups. This *ex vivo* reactivation [redacted] model is now established and routinely used in our lab (Ref [1] and **Figs. 3** to **12**). ***Subtypes of sensory neurons:*** Previous studies in [redacted] by Dr. Bertke have shown that HSV preferentially replicate and establish latency in different subtypes of sensory neurons[6, 7, 102-106]. We will therefore stain for $CD4^+$ and $CD8^+$ T cells together with Fe-A5 mAb ($A5^+$) and isolectin IB4 mAb ($KH10^+$) and determine by confocal microscopy whether different subtypes of sensory neurons are co-localized with DRG-resident $CD4^+$ and $CD8^+$ T cells. Finally, by localizing T cells around the neuronal cell body in DRG and around the axon termini that extend in VMC, we will determine whether different phenotypes of $CD4^+$ and $CD8^+$ T cells are co-localized with the cell body of sensor neurons vs. the axon ends (as illustrated in **Fig. 9**).

Figure 8. Blockade of PD-L1 significantly reduces virus reactivation in **DRG** explants in theHSV-2 infected [redacted]

Immune checkpoints blockade: HSV-2 is able to escape T cell surveillance by inducing phenotypic and functional exhaustion of $CD4^+$ and $CD8^+$ T cell responses to successfully coexist with the host and establish long-term latency. We recently discovered that increased frequency of PD-1 and LAG-3-positive $CD4^+$ and $CD8^+$ T cells in the VMC and DRG of HSV-2-infected [redacted] correlated with a lack of protection[1]. We have shown that the mAbs anti-PDL-1 decreased virus reactivation *ex vivo* in [redacted] DRG explant, suggesting a reversion of T cells exhaustion (**Fig. 8**)**.** PD-1 and LAG-3 pathway will be blocked *in vivo* following treatment with specific mAbs or peptide inhibitors on days 3, 5, 7 post infection (acute phase) and then on days 37, 39, 41 post infection (latent phase), as we recently demonstrated[107] (**Fig. 7B**). This will be performed in groups of [redacted] with less protective prime/pull vaccine to see whether immune checkpoint would improve protection. ***Statistical Analyses:*** Statistics will be performed with the help of our collaborator Dr. McLaren, a UCI biostatistician (see letter). Briefly, virological, immunological, transcriptional, and disease parameters will be determined in every [redacted] from the same group. We will apply a Generalized Estimating Equations (GEE) approach to analyze the Time-Average Difference (TAD) in means of severity scores between the vaccinated groups (Group 1 to Group 18 in **Table 1**) and the negative control groups (Group 19 to Group 32)[108, 109]. For each group, based on preliminary evidence, it is assumed that a sample of two [redacted] will develop severe recurrent genital herpes infection or disease, each measured on days 35 to 55 for a total of 20 measurements. This will achieve a power of at least 0.90 using a two-sided Wald test from a GEE analysis to test whether the TAD of the vaccinated [redacted] differs from that of the control [redacted] by more than 0.40 at a significance level of 0.05.

Expected results and interpretation: ***Safety:*** We expect the prime/pull vaccine to be safe and we recently demonstrated with CXCL10 in mice and rabbit models[110]. Besides, a recent malaria vaccine, using the SAPN delivery system, showed safety in Phase I/IIa clinical trials[63-73]. AAV8 vector is also safe clinical trials[97, 111]. ***Protection against genital herpes infection and disease:*** We expect at least one of the VP16, VP22, RR1 and/or RR2 prime/pull therapeutic vaccine candidates (Groups 1 to 18 in **Table 1**) to significantly reduce the frequency and/or magnitude of virus reactivation form DRG and viral shedding in VMC. Based on our experience with [redacted] model of recurrent herpes, the residual standard deviation is anticipated to be 0.40. We expect no or little HSV-2 DNA will be detected in any vaginal swab sample from the best prime/pull vaccinated [redacted] a result that would suggest that viral reactivation and/or shedding is significantly reduced by that prime/pull vaccine. This will be associated with a significant reduction of the frequency recurrent lesion outbreaks, to decrease the total lesion days per [redacted] and decrease lesion severity scores relative to the protein-based vaccinated counterpart group (n = 20) and relative to the mock-vaccinated control group (n = 20). We also expect the frequency with which prime/pull vaccinated [redacted] shed virus to be significantly reduced as compared to the protein-based vaccinated counterpart groups (Groups 25 to 30 in **Table 1**) mock-vaccinated control (group 32, in **Table 1**). This would suggest that a decrease in the number of days with recurrent lesions, lesion severity, and shedding may be due to enhanced antiviral DRG-tissue-resident $CD4^+$ and $CD8^+$ T cells that control reactivation. Thus, we expect at least one VP16, VP22, RR1 and/or RR2 prime/pull vaccine candidates (i.e., Groups 1 to 18 in **Table 1)** to significantly decrease viral loads in the DRG as compared to the gD control group (Groups 22 to 24 in **Table 1**). Although unlikely, it is possible that the prime/pull vaccine may prevent symptoms without preventing the underlying infection, causing more episodes of unrecognized or asymptomatic infection, or it may protect against neither disease nor infection. We expect the combination of protein with T-cell attracting chemokines to have an advantage over the protein vaccine alone. We expect our kinetic studies to

define the optimal frequency and function timing of HSV-specific $CD4^+$ and $CD8^+$ T_{RM} cells that develop in VMC and DRG of prime/pull vaccinated 6255. A reduction in the frequency by which 6255 shed virus after prime/pull vaccination with at least one of the 18 VP16, VP22, RR1 and/or RR2 prime/pull vaccine candidates (**Table 1**) would suggest that immune responses to that vaccine play a role in controlling viral reactivation from DRG. A decrease in the number of days with recurrent lesions, lesion severity, and shedding may also be due to an increase in number and function of tissue-resident $CD4^+$ and/or $CD8^+$ T cells that control reactivation in DRG and subsequent viral replication in VMC. ***Latency in DRG:*** We expect at least one prime/pull vaccine to decrease the load of latency in this DRG along the spinal column. ***Latency in SSG:*** We expect to confirm evidence of viral reactivation/latency in SSG, as recently reported by Dr. Krause[6]. In addition, we expect to confirm a low latency level in the SSG of the vaccinated group[6]. ***Longevity of protection:*** We expect at least one lead prime/pull vaccines (out of the first 18 groups in **Table 1**) to induce protection that lasts up to 12 months after vaccination. ***Subtypes of sensory neurons:*** We expect to find that: (***i***) antiviral DRG tissue-resident $CD4^+$ and $CD8^+$ T cells are localized around KH10 neurons, but not A5 neurons; and (***ii***) DRG tissue-resident $CD4^+$ and $CD8^+$ T cells and are both co-localized with the cell body of sensory neurons in DRG and the axon ends in VMC. ***Immune checkpoints blockade:*** We expect *in vivo* blockade of PD-1 and LAG-3 immune checkpoints with mAbs and/or peptide inhibitors to reduce virus reactivation and improve protection. This would suggest a reversion of would-be exhausted antiviral DRG-tissue resident $CD4^+$ and $CD8^+$ T cells that control reactivation, confirming our *ex vivo* finding (**Fig. 8**)

Pitfalls and Additional Considerations: No immunological, virological or transcriptome technical difficulties are expected since we are able to perform these techniques in the 6255 model[1]. ***Safety:*** No local or systemic side effects were noticed in the 6255 following expression of CXCL11 in VMC and DRG of HSV-2 infected 6255 AAV8 vector expressing the 6255 CXCL11 applied in the footpad did not cause any local inflammation. Long-term monitoring of the VMC and DRG for pathology associated with AAV8-induced inflammation will be performed to ensure the vectors' safety. ***Combination of SAPN antigens:*** Although unlikely, if no protection is provided with any of the 18 prime/pull vaccine candidates, a dose higher than 50 µg will also be tested. ***Combination of CXCL-9, -10, -11:*** If no protection is provided by single CXCL-9, -10, -11 chemokines, we will deliver combinations of 2 or 3 chemokines. ***HSV-2 clinical isolates:*** If time and resources allow, we will test the prime/pull vaccine against the less-virulent American clinical isolates (i.e., $186syn^+$-1 and 89-390) and the virulent African clinical isolates (i.e., SD90-3P, SD15, and SD66) (generously gifted by Dr. Knipe, Harvard University). ***Genital herpes caused by HSV-1:*** Besides HSV-2, HSV-1 has emerged as the cause of ~50% of genital herpes cases[113-115]. The lead prime/pull vaccine will also be tested in HSV-1-infected 6255 6255 ***Prophylactic vaccine:*** The lead prime/pull vaccine will be tested in a prophylactic setting (n = 20) to determine if it will also reduce primary acute genital herpes after virus challenge.

Specific Aim 2. To determine whether increase in the number and function of antiviral tissue-resident $CD4^+$ and $CD8^+$ T cells within: (1) DRG (central neuronal immunity); and (2) VMC (peripheral epithelial immunity) will correlate with protection against recurrent genital herpes infection and disease.

Rationale: Dorsal root ganglia (DRG) and vaginal muco-cutaneous (VMC) are the most obvious battlefield sites for the host's B- and T-cells to control recurrent genital herpes. Our preliminary phenotypic, functional, and transcriptomic T cell results (**Figs. 3-12**) obtained in the 6255 model of recurrent genital herpes revealed that: (**1**) Increased numbers of $CXCR3^+CD4^+$ and $CXCR3^+CD8^+$ T cells were detected in the VMC and DRG tissues of protected compared to non-protected 6255 (**Figs. 3** and **4C**); and (**2**) Similar to HSV-2 VP16-specific $CD8^+$ T cells in HSV-2 infected and asymptomatic women ((**Fig. 4A**), elevated levels of CXCR3 receptor and T-cell attracting CXCL9, CXCL10 and CXCL11 (ligands of CXCR3) were detected in $CD8^+$ T cells from DRG and VMC of HSV-2 infected and protected 6255 at both RNA transcript by RNA-Seq (**Fig. 4B**), and protein levels by immunostaining (**Fig. 4D**) and western blot (**Fig. 4E**), as compared to non-protected 6255 In Aim 2, we will use *in silico, in vitro*, *in situ, ex vivo*, and *in vivo* assays to shed more light on the relative contribution of the **central neuronal T cell immunity** (in the DRG) vs. **peripheral epithelial T cell immunity** (in the VMC) in protection against recurrent genital herpes (as illustrated in **Fig. 9**).

Figure 9. The prime/pull vaccine is expected to boost both the (**1**) **central neuronal immunity** (*left top panel*) and (**2**) **peripheral epithelial immunity** (*right top panel*). Black arrows illustrate levels of T-cell immunities.

Aim 2. 1. To determine T cell correlates of *central neuronal protective immunity:*

2. 1. 1. Experimental Design: ***Frequency:*** The DRG will be harvested from groups of protected and non-protected [redacted] that will be sacrificed before and 10, 15, 30, and 90-days post-immunization (*n* = 20/group, **Fig. 7B**). The number and function of HSV-specific memory $CD4^+$ and $CD8^+$ T cells within the DRG, their association with the level of latency and the frequency of virus reactivation from the DRG will be determined. The number and % of $CXCR3^{(+)}CD4^+$ and $CXCR3^{(+)}CD8^+$ T cells in DRG will be determined by FACS (**Figs. 3, 6**, **8, 10** and **11**). We will also determine the frequency of $CXCR3^{(+)}CD4^+$ and $CXCR3^{(+)}CD8^+$ T cells in HSV-2 DNA^{POS} vs. HSV-2 DNA^{NEG} DRG, as has been performed in human TG[116][117, 118]. We will determine the expression levels of CD69, CD103 (known as integrin α4β7), CCR7, CD62L (also known as L-selectin), T-bet, and Eomes. ***Location:*** We will define the anatomic localization of sensory ganglia-resident $CD4^+$ and $CD8^+$ T cells relative to individual HSV-infected neurons. With the help of our collaborator and imaging specialist, Dr. Jester (see letter), we will determine whether different subtypes of sensory neurons (i.e., $A5^+$ and $KH10^+$) are co-localized and targeted by $CD4^+$ and $CD8^+$ T cells. ***Function:*** We will determine functional properties of DRG-resident $CD4^+$ and $CD8^+$ T cells in prime/pull-vaccinated [redacted] that show rapid and effective peripheral control of HSV-2 with low shedding and fast-healing tissue. These functional properties will then be compared to those of mock-vaccinated and non-protected [redacted] Chemokine levels (both mRNA and protein) will be determined in DRG using RT-PCR and Luminex assays[3, 118-122]. HSV-specific $CD4^+$ and $CD8^+$ T cell function will be measured by IFN-γ ELISPOT assays, as we successfully done in our preliminary data (Ref [1] and **Fig. 12** below). $CD4^+$ and $CD8^+$ T cells' cytotoxic activity will be analyzed by $CD107^{a/b}$ degranulation assays[123]. We will also determine tissue-derived signals, including TNF-α, TGFβ, IL-15, and IL-33, which support $CD4^+$ and $CD8^+$ T_{RM} differentiation and survival in local tissue[124-127]. ***Single-cell scRNA-Seq:*** We will use single-cell RNA sequencing (scRNA-Seq) in protected and non-protected [redacted] as we successfully done in our preliminary data (**Fig. 4B**, above), to identify biomarkers predictive of protection and to define T cell pathways in sensory ganglia. We will profile DRG-resident HSV-specific $CD4^+$ and $CD8^+$ T cells longitudinally as they respond to vaccine and virus reactivation. On days 4, 7, 30, and 60 after vaccination of HSV-2-infected [redacted] $CD4^+$ and $CD8^+$ T cells will be sorted from the DRG and VMC. We will perform isolation of single-cell RNA and cDNA synthesis using the Fluidigm C1 Single-cell RNA sequencing integrated fluidic circuit and C1 Single-Cell Auto Prep System. Post-scRNA-Seq, reads will be mapped to the [redacted] genome (Cavpor3.0) using the spliced transcript aligner STAR35. ***Correlation*** between HSV-2 reactivation (virus shedding in genital tract) and recurrent genital herpetic disease with number and function of local $CD4^+$ and $CD8^+$ T_{RM} cells in DRG and SSG will be determined. We will leverage our recent experience on scRNA-Seq in the [redacted] model to determine correlation between protection and phenotypic and functional genes of tissue-resident $CD4^+$ and $CD8^+$ T cells in DRG. ***Ex vivo T cell depletion in explanted DRG:*** HSV-2 can be transmitted asymptomatically in the absence of genital lesions, allowing efficient virus spread amongst the general population[1]. Similar to humans, in the [redacted] model, recurrent genital lesions arise from spontaneous reactivation of latent virus in sensory neurons cell bodies of dorsal root ganglia (DRG)[1]. However, HSV-2 has also recently been found to reactivate from latently infected autonomic SSG[6]. This suggests that, in addition to sensory ganglia, HSV-2 reactivation from autonomic ganglia may also contribute to viral shedding in the genital tract and lead to recurrent genital herpes. However, local immune responses may also decrease local virus replication in the VMC tissue. We will assess HSV-2 reactivation directly *ex vivo* by explanting [redacted] DRG-induced reactivation (**Fig. 8**). DRG from latently infected prime/pull vaccinated [redacted] will be harvested at 7 and 21 days post-immunization. *Ex vivo* explant DRG will be cultured as is, or depleted *ex vivo* of $CD4^+$ and/or $CD8^+$ T cells as described[101]. We will use explant ganglia from the [redacted] at 10, 15, 30, and 90 days post-infection during the latent

Figure 10. Tissue-resident memory $CD4^+$ and $CD8^+$ T cells increased in the DRG of HSV-2 infected and protected [redacted] following treatment with CXCL11. Gated on $CD44^{pos}$ cells.

Figure 11. Tissue-resident memory T_{RM} cells and effector memory T_{EM} cells increased in the DRG of HSV-2 infected and protected [redacted] following treatment with CXCL11. Gated on $CD44^{pos}$ cells.

Figure 12. Frequent $IFN\gamma^{(+)}$ $CD8^+$ T cells detected in the DRG of HSV-2 infected and protected [redacted] following treatment with CXCL11.

phase of infection. Immunostaining of CXCR3$^{(+)}$CD4^{+} and CXCR3$^{(+)}$CD8^{+} T cells and their co-localization with infected neurons in DRG will be determined.

In vivo depletion of CD4^{+} and CD8^{+} T cells: To assess the involvement of CD4^{+} and CD8^{+} T cell subsets in the protection induced by a candidate vaccine, *in vivo* depletion of either CD4^{+} or CD8^{+} T cells using specific mAbs injected intravenously will be performed in infected 6255 before and after each immunization, as we previously described[1]. We have shown that CD4^{+} or CD8^{+} T cells not only infiltrate to the sites of infection, but also persist in DRG and VMC for prolonged time periods after viral clearance[1].

2. 1. 2. Expected Results and Interpretation:

Frequency: We expect at least one out of the 18 prime/pull vaccine candidates in **Table 1** to "pull" more protective local CD4^{+} and CD8^{+} T cells into the DRG compared to HSV-DL5-29-vaccine and to mock-vaccine, as illustrated in **Fig. 9**. We expect increased numbers and % of CXCR3$^{(+)}$CD4^{+} and CXCR3$^{(+)}$CD8^{+} T cells in DRG of prime/pull vaccinated protected 6255 consistent with mobility and attraction by local CXCL9, CXCL10 and CXCL11 chemokines. This would confirm the results that human ganglia that show CD8^{+} T cells express high levels of both CXCR3 and CCR5[119, 128]. We expect frequent CXCR3$^{(+)}$CD4^{+} and CXCR3$^{(+)}$CD8^{+} T cells to be present in HSV-2 DNA^{POS} vs. HSV-2 DNA^{NEG} DRG and SSG, suggesting attraction of HSV-specific T cells to infected ganglia. We also expect increased expression levels of CD69 and CD103, and decreased expression of receptors and transcription factors required for tissue egress and blood recirculation, such as CCR7, CD62L, S1PR1, KLRG1, T-bet, and Eomes, on CD4^{+} and CD8^{+} T cells in DRG of prime/pull infected 6255 suggesting molecular involvement in permanent retention of CD4^{+} and CD8^{+} T cells in peripheral DRG tissues. ***Location:*** We expect CD4^{+} and CD8^{+} T cells in prime/pull vaccinated protected 6255 but not in non-protected 6255 6255 to selectively cluster around both ends of infected neurons (as illustrated in **Fig. 9**). This could lead to efficient inhibition of virus reactivation from the infected neurons and a greater reduction in HSV shedding seen in vaccinated and protected 6255 [1]. Such a result would indicate that prime/pull vaccination that delivers T-cell attracting chemokines CXCL9, CXC10 and CXCL11 in infected neurons does "pull" vaccine-induced T cells closer to infected neurons, where they can react rapidly and abort any attempts at virus reactivation. We expect to find that antiviral CD4^{+} and CD8^{+} T cells are co-localized around KH10 neurons, but not A5 neurons, as previously shown in mice. ***Function:*** We expect high effector function of DRG-resident CD4^{+} and CD8^{+} T_{RM} cells, including increased expression of cytotoxic CD107$^{a/b}$ granules, antiviral IFN-γ and TNF-α cytokines, and T cell-attracting CXCL9, CXCL10 and CXCL11 chemokines to be associated with effective control of HSV-2 reactivation from DRG/SSG. We expect an increased number of HSV-specific IFN-γ-producing CD4^{+} and CD8^{+} T cells, and an increased number of CD107$^{a/b+}$CD4^{+} and CD107$^{a/b+}$CD8^{+} cytotoxic T cells in DRG/SSG of prime/pull vaccinated 6255 Using our ultra-sensitive FISH assay, we will measure expression of *in situ* RNA transcripts of Granzyme (GzmA, GzmB and GzmK) and perforin markers for cytotoxic function, IFN-γ, and CXCL9, CXCL10, and CXCL11 chemokines as representative genes for T cell-attracting factors. Finally, we expect upregulation of tissue-derived protective factors, including TGF-β, TNF-α, IL-15, and IL-33, which have been implicated in the process of T_{RM} differentiation and survival in peripheral tissues[124-127]. We expect an increase in the CD69, CD103, CD101, and CD49a receptors in the prime/pull vaccinated and protected 6255 which are implicated in sequestration of T_{RM} cells in infected tissues[129]. ***Single cell scRNA-Seq:*** We expect these results to provide important insights into the molecular mechanisms of antiviral central neuronal T cell immunity in DRG/SSG. We expect to identify biomarkers predictive of protection and to define immune pathways. ***Correlation:*** We expect CD4^{+} and CD8^{+} T cells in DRG of prime/pull vaccinated 6255 with elevated production of cytolytic granules, increased expression of antiviral cytokine/chemokine, and onsite proliferation. One of the hallmark features of memory CD4^{+} and CD8^{+} T_{RM} cells is their ability to perform immune surveillance and rapid clearance of re-encountered virus. Thus, more antiviral effector CD4^{+} and CD8^{+} T_{RM} cells retained in latently-infected DRG of prime/pull vaccinated 6255 should interfere with "attempts" or clear "ongoing" virus reactivations in neurons of DRG. This would translate into a reduction in subsequent virus shedding in the VMC and less recurrent genital herpes disease. We expect CD4^{+} and CD8^{+} T_{RM} cells to greatly outnumber recirculating T_{RM} and T_{CM} cells within non-lymphoid DRG and VMC tissues of protected 6255 [131]. Such results would: (**1**) reveal tissue compartmentalization as a major determining factor for immune-mediated protection against recurrent herpes; and (**2**) suggest that the temporal limitation on access of antiviral CD4^{+} and CD8^{+} T cells into the immunologically restricted DRG compartment is overcome with the prime/pull vaccine. We expect to shed light on whether T cell immunity in the autonomic ganglia has an important role in controlling viral reactivation recurrent disease. ***Ex vivo depletion of CD4^{+} and CD8^{+} T cells:*** We expect at least one out of the 18 prime/pull vaccine candidates in **Table 1** to significantly reduce HSV-2 reactivation in the *ex vivo* explant DRG-induced model. We also expect that *ex vivo* depletion of CD4^{+} and/or CD8^{+} T cells will increase virus reactivation in explanted DRG. ***In vivo depletion of CD4^{+} and CD8^{+} T cells:*** We expect the *in vivo* depletion of CD4^{+} and/or CD8^{+} T cells to abrogate the central and/or peripheral immunity induced by the prime/pull vaccine.

Aim 2. 2. To determine T cell correlates of *peripheral epithelial protective immunity:*

2. 2. 1. Experimental Design: ***Frequency:*** The frequency of $CD4^+$ and $CD8^+$ T cells that reside in the VMC of protected vs. non-protected 6255 will be quantified and 10, 15, 30, and 90 days post-immunization (n = 20/group, **Fig. 7B**). The location of $CD4^+$ and $CD8^+$ T cells will be determined at the dermal-epidermal junction (DEJ) of the epidermis. ***Function:*** Using the same markers as for central T cell immunity in DRG/SSG as above, we will determine the function and exhaustion of dermal-epidermal junction tissue-resident memory $CD4^+$ and $CD8^+$ T_{RM} cells in vaccinated and control 6255 The longevity of tissue-resident $CD4^+$ and $CD8^+$ T cell responses will be determined for up to 12 months. ***Single cell scRNA-Seq:*** Before and 10, 15, 30, and 90 days post-immunization, $CD4^+$ and $CD8^+$ T_{RM} cells will be sorted from the VMC. Similar to DRG/SG in Aim 2.1 above, and as successfully shown in our preliminary data (**Figs. 3A** to **3C**), we will use scRNA-Seq to identify biomarkers predictive of protection in the VMC and to define T cell pathways in VMC of protected 6255 and subsequently perform scRNA-Seq for DRG/SSG. ***Correlation:*** The number of $CD4^+$ and $CD8^+$ T cells will be correlated with rates and level of virus shedding and recurrent disease severity. The association of the HSV-specific $CD4^+$ and $CD8^+$ T cell responses, the frequency of virus shedding and replication in the VMC epithelial cells, and subsequent recurrent genital herpes will be determined. In addition, the longevity of tissue-resident $CD4^+$ and $CD8^+$ T cell responses that are associated with protection will be determined for up to 12 months after vaccination. ***Tissue-resident vs. circulating memory T cells in protection:*** We will determine whether increased number of T cells in VMC and DRG is due to: (1) a boost of a pre-existing pool of tissue-resident T_{RM} cells; or (2) circulating memory T cells (T_{CM} and T_{EM}) migrating toward T-cell attracting chemokines delivered locally. T_{CM}, T_{EM}, and T_{RM} can be detected in 6255 VMC and DRG. A separate group of prime/pull vaccinated latently infected 6255 (n = 20) will be treated with the fungal metabolite fingolimod FTY720, a CCR7 chemokine antagonist that blocks T-cell migration from circulation and regional lymph nodes to home in to peripheral inflamed tissues, such as VMC and DRG[132-134]. FTY720 will be given IP, 5 times, 1 day before AAV8-chemokines treatment and then every other day. ***Neutralizing antibodies*** from serum (IgG) and vaginal washes (IgA) will also be correlated with protection, as we reported[1]. ***Mathematical modeling:*** With the help of our collaborator Dr. Elizabeth Read (see letter), dynamical mathematical models will be designed to identify the relative contribution of the peripheral epithelial T cell immunity (in the VMC) vs. central neuronal T cell immunity (in the DRG) in the protection against recurrent genital herpes (as illustrated in **Fig. 9**).

2. 2. 2. Expected Results and **Interpretation:** ***Frequency:*** We expect at least 1 out of the 18 prime/pull vaccine candidates in **Table 1** to "pull" more protective local $CD4^+$ and $CD8^+$ T cells into the VMC, as illustrated in **Fig. 9**. We expect a high number of $CD4^+$ and $CD8^+$ T cells at the dermal-epidermal junction (DEJ) of protected 6255 with no lesions. ***Function:*** We expect 6255 with higher density of functional $CD4^+$ and $CD8^+$ T cells in the epidermis to have a lower shedding rate. We expect increased expression of cytotoxic $CD107^{a/b}$ granules, antiviral IFN-γ and TNF-α cytokines to be associated with low shedding rate. We expect at least one prime/pull vaccine candidate will induce tissue-resident $CD4^+$ and $CD8^+$ T cell-dependent protection that will last up to 12 months after vaccination. ***scRNA-Seq:*** We expect to identify T cell pathways in the VMC epithelium barrier that associated with peripheral epithelial immunity. ***Correlation:*** We expect prime/pull vaccinated and protected 6255 to present low virus shedding rates and healed VMC tissue that will correlate with frequent VMC-resident $CD4^+$ and $CD8^+$ T cells. ***Tissue-resident vs. circulating memory T cells in protection:*** We expect FTY720 treatment to significantly reduce the % and N^{br} of $CD4^+$ and $CD8^+$ T cells within the DRG and VMC and abrogate protection induced by the prime/pull vaccine. This would suggest the importance of circulating T cells (i.e. T_{CM} and T_{EM}) migrating toward the T-cell-attracting chemokines delivered locally in infected DRG and VMC. ***Neutralizing antibodies:*** It is likely that the prime/pull vaccine induce neutralizing antibodies (in addition to T cells). ***Mathematical modeling:*** We expect mathematical modeling to shed light the relative contribution of the peripheral epithelial immunity vs. central neuronal immunity in protection.

2. 2. 3. Pitfalls and **Additional Considerations**: Aim 2 does not depend on the success of Aim 1, because we previously showed protection by the replication-defective HSV-2 dl5-29 vaccine[1]. The dl5-29 infects the VMC epithelium, but do not develop latency in DRG, and hence, the HSV-2 dl5-29 mainly induce peripheral epithelial immunity[1, 135-137]. Thus, regardless of protection in Aim 1, we will still be able to compare the role of peripheral epithelial immunity and central neuronal immunity in protection. We will also determine whether the prime/pull vaccine will attract NK, NKT cells, and MΦ (besides T cells) to the VMC and DRG[87, 89, 130, 138, 139].

Timetable: Year 1: Complete Aim 1, vaccine candidates 1 to 5, begin Aim 1, vaccine candidates 6 to 10 .**Year 2:** Complete Aim 1, vaccine candidates 6 to 10, begin Aim 1, vaccine candidates 11 to 18. **Year 3:** Complete Aim 1, vaccine candidates 11 to 18, begin Aim 2. **Year 4:** Complete Aims 2 central immunity, begin Aim 2 peripheral immunity. **Year 5:** Complete Aim 2 .

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	❍ Yes ● No
Other Requested information	

Pages 95 through 98 redacted for the following reasons:
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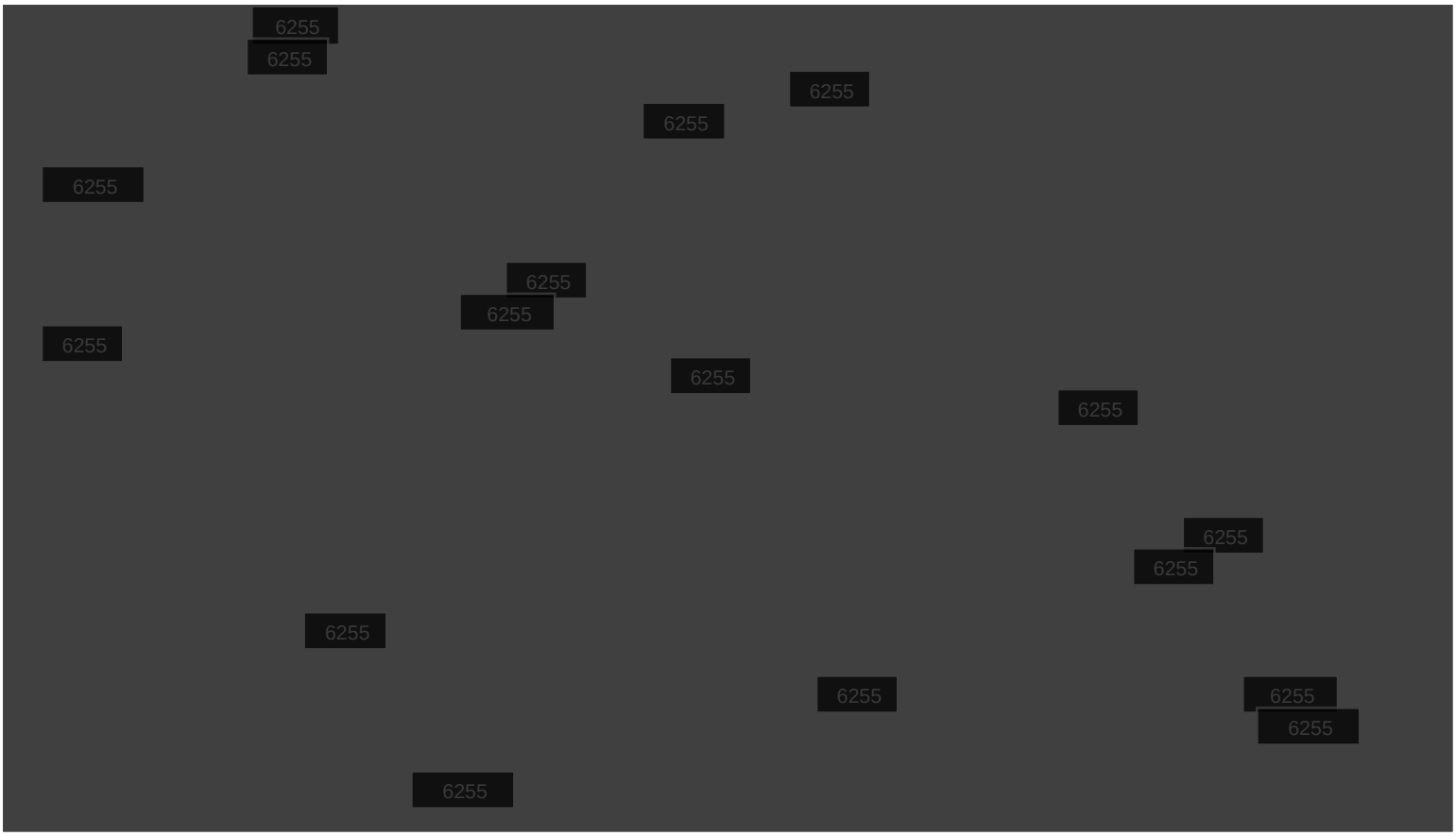
BIOHAZARDS

Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2) are a biosafety level 2 (BSL-2) biohazard. All HSV-1 and HSV-2 6255 work done in our vivarium is performed using ABSL-2 level precautions. The BSL-2 and ABSL-2 guidelines are designed to protect the research personnel and the environment from HSV infection. The IBC and the IACUC committees, which meet monthly at UC Irvine, have approved all laboratory and 6255 procedures involving HSV in this proposal (protocol # 2009-1304 and 2002-2372, respectively).

Laboratory environment and safety: Our laboratory is equipped for BSL-2 research and certified by UC Irvine Environmental Health and Safety and the IBC. HSV is handled under biosafety level 2 conditions using BSL-2 rated biosafety cabinets.

As indicated in our IBC protocol, all virus work in the lab is done in a specific room (room 2337) within our main lab. This “virus” room is dedicated to HSV work. For work involving infection of tissue culture cells with HSV, “clean” cells are brought to room 2337 from our clean tissue culture room (just a few yards away) and used in the BSL-2 certified Biosafety cabinets dedicated to virus work. Infected cell cultures are cultures in CO2 incubators in the same room. Personal Protective Equipment (PPE) for work in a Biosafety hood includes lab coat, eye protection, and gloves. All surfaces are disinfected with 10% bleach after use. All HSV liquid waste is incubated in a 10% solution (final concentration) of bleach for at least 30 minutes and then disposed of down a drain. Solid HSV waste (plasticware) is disposed of as solid Medical Waste in properly labeled biohazard waste containers and collected by Environmental Health & Safety. The approved protocols are updated and renewed every year. Our lab is (as are all the labs in our building) subject to regular inspection by Environmental Health & Safety, IBC, and various state agencies, to assure compliance with all regulations regarding the use of biohazardous agents and infectious waste. All lab personnel have to take various biosafety courses, including “blood borne pathogens”, and receive specific HSV safety training by a senior level individual in the lab, prior to working with HSV. The door to the lab as well as the specific HSV room has a biohazard warning label indicating that HSV is used inside.



Personnel training: Dr. BenMohamed (PI) has over 15 years’ experience utilizing HSV-1 and HSV in

laboratory settings. All laboratory workers, including the PI, who work with 6255 or HSV are required to take yearly specific training in laboratory safety at UCI including: (*i*) Wearing proper PPE (gloves, eye protection, labcoat etc...) when manipulating HSV, HSV-infected tissues HSV-infected 6255 (*ii*) proper virological techniques; (*iii*) the proper use and disposal of biohazardous agents and infectious waste into biohazardous waste containers; (*iv*) biohazard training; (*v*) Aerosol Transmissible Diseases (ATD) training; (*vi*) medical waste handling; and (*vii*) shipment of biohazardous materials. All new personnel are handed Standard Operating Procedures (SOP) and trained upon arrival and then on an annual basis. Research support services include training classes; seminars and wet labs are offered at UCI throughout the year.

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CONSORTIUM/CONTRACTUAL ARRANGEMENTS

The appropriate programmatic and administrative personnel between UCI and Sunomix Therapeutics. Inc. are aware of the agency's consortium agreement policy and are prepared to establish the necessary inter-organizational agreement(s) consistent with that policy.

Sunomix Therapeutics, Inc. (San Diego) is specialized in making the Self-Assembling Protein Nanoparticles (SAPNs) and will develop and provide UC Irvine these nanoparticles for during the 5-years of this project.

Peter Burkhard, Ph.D. will be directly involved in the design, development, cloning, protein expression, purification, refolding, TLR-activation and analysis of the gD, VP16, VP22 RR1 or RR2 proteins-based SAPNs. The Vaccine will then be tested in the 6255 at UC Irvine.

The prototypes will be send to Sunomix for scale up production. Vaccination with immunodominant single or multiple HSV-2 proteins incorporated in SAPNs vaccines will be done at UC Irvine and is expected to induce a broad and strong T cell responses. This multi-component vaccine can elicit multi-faceted immune responses, providing protections against recurrent genital herpes infection and disease in the 6255 model of genital herpes. This will involve testing safety, immunogenicity and protective efficacy of multiple combinations of SAPNs-based prime/pull vaccines in 6255 model.

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Please reply to:

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April 3rd, 2019

Dr. Lbachir BenMohamed, PhD.
Professor & Director
Cellular & Molecular Immunology Laboratory
The Gavin S. Herbert Eye Institute & Center for Immunology
UC Irvine, School of Medicine
Hewitt Hall, Room 232
843 Health Sciences Rd
Irvine, CA 92697-4390

Dear Lbachir,

I am writing to confirm that I will collaborate on your new vaccine grant proposal entitled "*A Novel Prime/Pull Therapeutic Vaccine Strategy to Prevent Recurrent Genital Herpes*" to be submitted to the National Institute of Allergy and Infectious Disease.

I look forward to providing expertise in biostatistics with regard to study design, monitoring, and data analysis protective efficacy results obtained in 6255 following prime/pull vaccination and the various treatments that you propose to block the co-inhibitory pathways.

I believe that your novel therapeutic prime/pull vaccine strategy has the potential to provide protection from genital herpes.

I look forward to our collaborative work on this project.

Sincerely,

Christine E. McLaren

Christine E. McLaren, Ph.D.
Vice Chair and Professor, Department of Epidemiology
Director of Biostatistics

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Lbachir BenMohamed, PhD.
Professor & Director

Cellular & Molecular Immunology Laboratory
The Gavin Herbert Institute
Institute for Immunology
UC Irvine, School of Medicine
University of California Irvine

Riehen, March 27, 2017

Dear Dr. BenMohamed,

As you know, Alpha-O peptides owns valuable technology, intellectual property (US8575110, US8546337, US2014/0242104A1, EP3092245A1, and EP17157687.9) and proprietary information related to the design, construction, and bio-production of self-assembling protein nanoparticles (SAPNs). For this project of engineering ten pairs of CD4/CD8 T-cell epitopes into the SAPNs, Alpha-O Peptides has executed a separate written agreement to work exclusively with Sunomix Therapeutics and to transfer the technology for the bio-production of such SAPN-based HSV-vaccines from Alpha-O Peptides to Sunomix Therapeutics. Alpha-O Peptides will provide Sunomix Therapeutics at least 2 mg of pure protein of two SAPN-constructs. Sunomix Therapeutics will use the validated bio-production protocol provided by Alpha-O to generate nine similar HSV-SAPN constructs. Sunomix Therapeutics will be responsible to deliver at least 2 mg of pure SAPN-protein of eleven constructs (ten vaccine constructs and one negative control) to your laboratory at the University of California, Irvine, to be used for the immunization experiments of the project described in the NIH-STTR genital herpes vaccine grant proposal.
The experiments you propose to test the SAPN-based genital herpes vaccines in “humanized” HLA trangenic mice are very innovative. I do not know of anyone conducting detailed studies of SAPN-based genital herpes vaccine. Based on your recent publications, it seems quite possible that T cells from HSV positive symptomatic vs. asymptomatic individuals recognize different sets of HSV antigens. Therefore, to develop a better herpes vaccine, I think it is crucial to answer the questions you are raising in your proposal.

Yours sincerely,

Peter Burkhard

CEO, Alpha-O Peptides AG

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April 5th, 2019

Lbachir BenMohamed, PhD
Professor/Director
University of California Irvine

Dear Lbachir,

I am very excited at the prospect of investigating the new vaccine strategy **"A Novel Prime/Pull Therapeutic Vaccine Strategy to Prevent Recurrent Genital Herpes "** described in your new R01 grant proposal to the NIH.

The new prime/pull vaccine strategy proposed in this new R01 vaccine project is expected to induce more tissue resident HSV-specific $CD4^+$ and $CD8^+$ T cells in VMC and DRG.

Your proposal, which bridges contemporary virology and immunology is innovative.

We will employ intracellular immunohistochemistry to identify $CD4^+$ and $CD8^+$ T cells within frozen sections of infected and uninfected vaginal mucosa and DRG tissues in conjunction with surface staining for leukocyte population markers.

We will also use several VMC and DRG sections (~2um each) and a novel Multiplexed High-Resolution Macroscopy technique for 3D reconstruction of VMC and DRG sections. This will allow us to determine co-localization of HSV-infected neurons with $CD4^+$ and $CD8^+$ T cells three-dimensionally in and at high-resolution on a macroscopic scale.

I look forward to a fruitful collaboration on this exciting project

Warm Regards,

James V. Jester, Ph.D.
Jack H. Skirball Endowed Research Chair
Professor of Ophthalmology and Biomedical Engineering

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Datum
04.04.2019

Dr. Hubert Schäfer
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Robert Koch-Institut
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www.rki.de

Dear Lbachir,

I am writing to confirm that I will collaborate on your vaccine grant proposal entitled "*A Novel Prime/Pull Therapeutic Vaccine Strategy to Prevent Recurrent Genital Herpes*" in which you are using the 6255 recurrent genital herpes model.

In the last 2-3 years, we have advanced the immunology frontiers in 6255 We have developed cutting-edge assays for phenotypic and functional characterization of circulating and tissue-resident $CD4^+$ and $CD8^+$ T cells of the 6255 model as attested in our 2019 *Journal of Virology* publication.

I believe that your proposed therapeutic prime/pull vaccine strategy has the potential to provide protection from genital herpes.

I look forward to continue this collaboration and provide my expertise and reagents to advance further the 6255 immunology

Sincerely,

Hubert Schaefer, PhD

Besucheranschriften
Nordufer 20
13353 Berlin

Das Robert Koch-Institut ist ein Bundesinstitut im Geschäftsbereich des Bundesministeriums für Gesundheit

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April 4, 2019

Lbachir BenMohamed, PhD
Professor & Director
Cellular & Molecular Immunology Laboratory

Dear Dr. BenMohamed,

We look forward to working with you on your plan to use single cells scRNASeq technology to analyze gene expression in 6255 tissue-resident $CD4^+$ and $CD8^+$ T cells, as described in your new grant proposal titled "*A Novel Prime/Pull Therapeutic Vaccine Strategy to Prevent Recurrent Genital **Herpes***".

The UC Irvine Genomic High-Throughput Facility (GHTF) currently offering preparation of mRNA, small RNA, genomic and exome sequencing libraries. The staff has experience with different strategies for exome enrichment, ribosomal RNA depletion and generation of multiplex libraries in order to insure maximal return of data. We also offer services that target applications for single cell genomics, using the Fluidigm C1 single cell sample prep platform and the 10x Genomics Chromium platform. The GHTF is equipped with Covaris S220 focused ultrasonicator; NanoDrop 1000 Spectrophotometer, MJ Research Tetrad thermal cycler, Agilent 2100 Bioanalyzer, Sage Biosciences Blue Pippin and Agilent MX PRO RTPCR to facilitate the preparation of libraries and quality testing and final titration of samples. We have the Illumina HiSeq 2500 with a dual mode system for high output or rapid mode sequencing, the Illumina HiSeq 4000 and the MiSeq. We also offer Pac Bio Sequel sequencing which provides very long read lengths that are useful for whole genome assembly, targeted sequencing, full length transcriptomes and for studying epigenetic modifications.

GHTF facility has all the necessary equipment required (Affymetrix GeneChip System-Scanner 3000 7G, Fluidics 450 and Hybridization 640 stations; MJ Research Tetrad thermacycler and Agilent 2100 Bioanalyzer), along with the requisite expertise for start-to-finish sample processing for either Affymetrix based Expression, SNP array analysis or from fragmentation-labeling step onward for Chip-on-Chip analysis. GCOS and Expression & GenoTyping Console (Affymetrix), Genomics Workbench (CLC Bio) and JMP Genomics (SAS) software programs are available for data analysis.

A dedicated bioinformaticist, Jenny Wu, Ph.D. is available to assist you with data analysis of your single cells scRNASeq experiments in the 6255 $CD4^+$ and $CD8^+$ T cells projects. Dr. Wu has experience with microarray and next generation sequencing data analysis including feature clustering, statistical analysis of gene expression, pathway analysis, exome-sequencing data analysis and disease causing gene identification and RNA-seq data analysis for differential expression for bulk and single cell RNA inputs.

We are therefore well equipped and excited to be involved in your single cells scRNASeq experiments in 6255 $CD4^+$ and $CD8^+$ T cells as proposed in your new prime/pull therapeutic vaccine project.

Best regards,

Melanie L. Oakes, Ph.D.

Manager
UCI Genomic High-Throughput Facility

Suzanne Sandmeyer, Ph.D.

Director
UCI Genomic High-Throughput Facility
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April 4th , 2019

Lbachir BenMohamed, PhD
Associate Professor & Director
Cellular & Molecular Immunology Laboratory
The Gavin Herbert Eye Institute
University of California, Irvine

Dear Dr. BenMohamed,

I look forward to working with you on your plan for single cell scRNA Seq analysis experiments in the 6255 model as described in in your new grant proposal titled "*A Novel Prime/Pull Therapeutic Vaccine Strategy to Prevent Recurrent Genital Herpes*"

The proposed research will make use of scRNASeq technology to compare the transcriptomes of tissue resident $CD4^+$ and $CD8^+$ T cells in the 6255 model of recurrent genital herpetic disease.

I will provide my experience with microarray and next generation sequencing data analysis including feature clustering, statistical analysis of gene expression in bulk and single cell RNA-seq. I am familiar with with standard single cell RNA-seq data analysis tools such as *Seurat,Scater, Scanpy, Monocle* and have experience with the newly developed tools such as *Velocyto* for RNA velocity analysis and *scCOGAPS/projectR* for data integration and batch correction. I have a demonstrated record in computational biology and bioinformatics software development.

I look forward to supporting your research endeavors.

Best regards,

Jenny Wu, Ph.D.

Director of Bioinformatics
UCI Genomic High-Throughput Facility

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April 2nd, 2019

Dr. Lbachir BenMohamed, PhD.
Professor & Director
Cellular & Molecular Immunology Laboratory
The Gavin S. Herbert Institute & Center for Immunology
UC Irvine, School of Medicine
Hewitt Hall, Room 232
843 Health Sciences Rd
Irvine, CA 92697-4390

Dear Lbachir,

I am writing to confirm my enthusiasm to collaborate on your new NIH RO1 grant proposal investigating novel prime/pull approach to boost functional HSV-specific $CD4^+$ and $CD8^+$ T cells in the sensory ganglia and the genital tract.

My work is in mathematical modeling applied to various areas of biology, including to virus-immune dynamics in chronic infection. My previous work in modeling $CD8^+$ T-cell dynamics, and my ongoing research interests in how T-cell population dynamics are shaped by complex signals in the context of viral infections, fit nicely with your project. My group can contribute to this effort by developing mathematical models of the $CD8^+$ T-cell response to genital HSV-1 and HSV-2, including how checkpoint blockade modulates the response. These models will be developed, tested, and refined iteratively on the basis of your experimental data. In this way, the mathematical modeling can help to inform rational strategies for combination therapies.

Your published work and data on DRG and VMC tissue-resident $CD4^+$ and $CD8^+$ T-cells in HSV-2 infected and protected 6255 indicate that therapeutic immunization that boost the number and function of $CD4^+$ and $CD8^+$ T-cells in these 2 compartments would protect from genital herpes. The prime/pull vaccine project you are proposing in your application should help understand the mechanisms by which T cell can be induced to better protect against blinding genital herpes. This work will help pave the way towards effective treatment of genital herpes.

I look forward to contributing to this research program.

Best wishes,

Elizabeth L. Read

Elizabeth Read, Ph. D.
Department of Chemical and Biomolecular Engineering
Department of Molecular Biology & Biochemistry
University of California, Irvine

RESOURCE SHARING PLAN

1. Data Sharing Plan: Data sharing is not applicable to this proposal.
2. Sharing Model Organisms: We will teach and share the 6255 model of recurrent genital herpes infection and extraction/studies of sensory ganglia-resident $CD4^+$ and $CD8^+$ T cells to any interested researcher globally.
3. Genome Wide Association Studies: Not applicable.

AUTHENTICATION OF KEY BIOLOGICAL AND CHEMICAL RESOURCES

- Although the state of the art in 6255 immunology still lags behind that of the 6255 and human, immunology several monoclonal and polyclonal antibodies specific to 6255 immune cell CD markers, cytokines and growth factors are now available in our lab.
- The identity and validity of key biological and chemical resources used in the proposed 6255 immunological and virological studies are published in our recent 2019 *Journal Virology* paper(1).
- These include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics.
- Unfortunately, we and others have already experienced that not all commercially available anti 6255 6255 antibodies work as they claimed. During the past five years we have dedicated a lot of effort to determine which reagents are useful for studying the 6255 immune system. We now have a tested panel of antibodies specific to 6255 immune cells, allowing for the unprecedented opportunity to assess the induction of 6255 6255 $CD4^+$ and $CD8^+$ T cells and their deployment in DRG and VMC to decrease HSV-2 spontaneous reactivation, and ultimately reduce or eliminate recurrent genital disease.
- Anti-6255 $CD4^+$ and $CD8^+$ T cell mAbs have already allowed us to analyze HSV-specific $CD4^+$ and $CD8^+$ T_{CM}, T_{EM} and T_{RM} cell infiltrates in DRG and VMC of infected 6255 (*Preliminary Results*).
- Finally, the P.I. recently formed a 6255 *Immunology Club* that gathers over twenty national and international laboratories using 6255 as an 6255 model in immunological research.
- We will share and teach the method of HSV1 and HSV-2 intravaginal infection, reading of recurrent genital herpetic disease, HSV reactivation from DRG explants and the extraction/studies of the vaginal mucocutaneous tissue and DRG resident $CD4^+$ and $CD8^+$ T cells in the 6255 model to any interested researcher. Parts of these experiments are also published in our *Journal Virology* paper (2019) (1).
- All the non-commercial reagents developed and used in the 6255 genital herpes model (e.g. mAbs used for *in vitro* phenotypic and functional characterization of $CD4^+$ and $CD8^+$ and mAbs used for *in vivo* $CD4^+$ and $CD8^+$ depletion) will be made available to other NIH funded researchers *via* applicable University of California Irvine Material Transfer Agreements and/or licensing agreements through the UCI Patents and Technology Transfer Department.
- No cell lines are used in this proposal. Standard laboratory reagents are not expected to vary, such as buffer and other common biologicals or chemicals.
- All the remaining reagents, cell lines and methods used in this proposal are detailed in our published papers.